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Echiniscidae in the Mascarenes: the wonders of Mauritius

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Abstract

Many regions of the world remain unexplored in terms of the tardigrade diversity, and the islands of the Indian Ocean are no exception. In this work, we report four species of the family Echiniscidae representing three genera from Mauritius, the second largest island in the Mascarene Archipelago. Two species belong in the genus *Echiniscus*: *Echiniscus perarmatus* Murray, 1907, a pantropical species, and one new species: *Echiniscus insularis* **sp. nov.**, one of the smallest members of the *spinulosus* group and the entire genus, being particularly interesting due to the presence of males and supernumerary teeth-like spicules along the margins of the dorsal plates. The new species most closely resembles *Echiniscus tropicalis* Binda & Pilato, 1995, for which we present extensive multipopulation data and greatly extend its distribution eastwards towards islands of Southeast Asia. *Pseudechiniscus* (*Meridioniscus*) *mascarenensis* **sp. nov.** is a typical member of the subgenus with elongated (dactyloid) cephalic papillae and the pseudosegmental plate IV' with reduced posterior projections in males. Finally, a *Bryodelphax* specimen is also recorded. The assemblage of both presumably endemic and widely distributed tardigrade species in Mauritius fits the recent emerging biogeographic patterns for this group of micrometazoans.

Key Words

Biodiversity, distribution, Heterotardigrada, insular fauna, morphology, sculpturing

Introduction

Tardigrades, as many micrometazoan taxa, remain mostly ignored in biodiversity surveys, although molecular techniques indicate the presence of multiple lineages and high potential for cryptic speciation (Blaxter et al. 2003, Cesari et al. 2020). Recent estimates augment the increasing evidence for the existence of numerous species complexes (Faurby et al. 2012; Jørgensen et al. 2018; Guidetti et al. 2019; Morek and Michalczyk 2020), however, rather mediocre species richness of this phylum emerges when compared to other animal groups characterised by greater species abundance by an order of magnitude (Bartels et al. 2016). Many regions of the world have never been sampled in a search for tardigrades, although the collection of these animals is very easy and not costly (Degma 2018). Archipelagos in the Western Indian Ocean are known as centres of insular endemism and local biodiversity hotspots (Goodman and Benstead 2005; Cheke and Hume 2008), but the scarcity of faunistic tardigrade studies precludes a more in-depth look into the evolutionary history of the phylum in this area. Tardigrades were particularly intensively sampled in the Seychelles (Biserov 1994; Binda and Pilato 1995; Pilato et al. 2002, 2004, 2006, 2009a, 2009b) and Madagascar (see Gąsiorek and Vončina 2019; Kaczmarek et al. 2020 for summary). Single papers were devoted to either limno-terrestrial or marine tardigrades of Mauritius (Grimaldi De Zio et al. 1987), Maldives (De Zio Grimaldi et al. 1999), and Réunion (Séméria 2003). Other archipelagos, like the Comoros or Socotra, have not been explored for these animals. Among the smaller islands of the Western Indian Ocean, the fauna of Mauritius has received the greatest attention and appeals for conservation effort of the best-studied insects, especially beetles (Motala et al. 2007).

The purpose of this contribution is to provide the first integrative data for the Mauritian members of the armoured tardigrades from the family Echiniscidae (Heterotardigrada). They include detailed DNA barcoding and morphological information for two species new to science, extracted from two moss samples. The new species represent the genera *Echiniscus* and *Pseudechiniscus*, the most speciose echiniscid taxa. Novel morphological characters are depicted for the *Echiniscus spinulosus* complex based on the smallest and dioecious member of this inordinately species-rich, by tardigrade standards, group. We also elaborate on *Echiniscus tropicalis*, the cognate taxon of the new species. Finally, the records of species with wide tropical or even pantropical distribution support the supposition that very broad geographic ranges may be typical for tropical tardigrade taxa (Gąsiorek et al. 2019). This is in accordance with data for oribatid mites inhabiting the Madagascan region, a significant fraction of which comprises pantropical species (Niedbała 2017).

Materials and methods

Sample collection and processing

Tardigrades were extracted from two moss samples (MU.001–2) collected from Sophie Nature Walk in the vicinity of Mare aux Vacoas (ca. 20°22'S, 57°29'E, 580 m asl; Mauritius, Mascarene Archipelago, Western Indian Ocean; O. Garmish leg. on 7th September 2019). Samples were rehydrated in Petri dishes, and then processed according to standard protocols (Dastych 1980; Stec et al. 2015). The animals were used in three analyses: (I) qualitative and quantitative morphology investigated with phase contrast microscopy (PCM); (II) high-resolution imaging with scanning electron microscopy (SEM); (III) DNA sequencing. Additionally, populations of *Echiniscus tropicalis* were obtained and underwent an identical procedure (Table 1).

Microscopy, imaging and morphometrics

Permanent microscope slides were made using Hoyer's medium and examined under Olympus BX53 phase contrast microscope (PCM) equipped with a digital camera Olympus DP74. In order to obtain ideally dorso-ventrally or dorso-laterally positioned and flattened specimens, specimens were first completely air-dried, then mounted in a minuscule drop of medium which did not fill the entire space between the slide and cover slip, and, eventually, the missing portion of medium was added at the edges of the cover slip to refill the empty space after 30 minutes. Specimens were prepared for SEM according to the protocol by Stec et al. (2015). All figures were assembled in Corel Photo-Paint X7. All measurements are given in micrometres (µm) and were performed under PCM. Structures were measured only when not broken, deformed or twisted, and their orientations were suitable. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. The *sp* ratio, the ratio of the length of a given structure to the length of the scapular plate, was expressed as a percentage (Dastych 1999). Morphometric data were handled using the Echiniscoidea ver. 1.3 template available from the Tardigrada Register, www.tardigrada.net (Michalczyk and Kaczmarek 2013). Raw data are presented as Suppl. materials 1–5. Scientific drawing of the ventral sculpturing pattern of the new *Pseudechiniscus* species was made in Microsoft PowerPoint using microphotographs and direct observations of specimens in PCM.

Genotyping, genetic comparisons and phylogenetics

DNA was extracted from individual animals following the Chelex 100 resin (Bio-Rad) extraction method (Casquet et al. 2012; Stec et al. 2015). Each specimen was observed in a drop of distilled water on a temporary slide under a 400× magnification prior to investigation. Hologenophores (Pleijel et al. 2008) were obtained for *E. insularis* sp. nov., *E. perarmatus* and *P. mascarenensis* sp. nov. Five

Table 1. List of the populations of *Echiniscus tropicalis* examined in this study. Types of analyses: (LCM) imaging and morphometry in PCM, (SEM) imaging in SEM, (DNA) DNA sequencing. Number in each analysis indicates how many specimens were utilised in a given method (a – adults, j – juveniles, l – larvae).

Sample code	Coordinates altitude	Locality	Sample type	Collector	Analyses		
					LCM	SEM	DNA
ID.032	8°16'35"S, 115°29'29"E, 521 m asl	Indonesia, Bali, Karangasem Regency	moss from tree bark	Łukasz Michalczyk	23a	20a	10a
ID.071	ca. 2°10'N, 97°26'E, 0–20 m asl	Indonesia, coastline of Sumatra, Palambak Island	moss from tree bark	Łukasz Skoczylas	3a	–	–
ID.858	0°39'47"N, 127°24'11"E, 1717 m asl	Indonesia, the Moluccas, Tidore, Gunung Kiematubu	moss and lichen from rock	Piotr Gąsiorek	2a	–	–
ID.939	1°15'53"N, 124°53'57"E, 696 m asl	Indonesia, Celebes, Sulawesi Utara, shores of Danau Tondano	moss and lichen from tree bark	Piotr Gąsiorek and Łukasz Krzywański	363a + 16j + 10l	10a	10a
ID.951	1°10'02"N, 124°49'22"E, 743 m asl	Indonesia, Celebes, Sulawesi Utara, Ramo Lewo	moss and lichen from palm tree	Piotr Gąsiorek and Łukasz Krzywański	1a	–	–
MY.008	5°58'54"N, 116°04'42"E, 30 m asl	Malaysia, Borneo, Sabah, Kota Kinabalu, Bukit Bendera Street	moss from concrete wall	Piotr Gąsiorek	1a + 1j	–	–
SG.001	1°21'39"N, 103°53'24"E, 12 m asl	Singapore	moss from tree bark	Tan Pal Chun	9a	–	–

Table 2. Primers and references for specific protocols for amplification of the four DNA fragments sequenced in the study.

DNA fragment	Primer name	Primer direction	Primer sequence (5'-3')	Primer source	PCR programme*
18S rRNA	18S_Tar_Ff1	forward	AGGCGAAACCGCGAATGGCTC	Stec et al. (2017)	Zeller (2010)
	18S_Tar_Rr2	reverse	CTGATCGCCTTCGAACCTCTAACTTTCG	Gąsiorek et al. (2017)	
28S rRNA	28S_Eutar_F	forward	ACCCGCTGAACTTAAGCATAT	Gąsiorek et al. (2018)	Mironov et al. (2012)
	28SR0990	reverse	CCTTGGTCCGTGTTTCAAGAC	Mironov et al. (2012)	
ITS-1	ITS1_Echi_F	forward	CCGTCGCTACTACCGATTGG	Gąsiorek et al. (2019)	Wełnicz et al. (2011)
	ITS1_Echi_R	reverse	GTTCAGAAAACCCTGCAATTCACG		
ITS-2	ITS-3	forward	GCATCGATGAAGAACGCAGC	White et al. (1990)	Wełnicz et al. (2011)
	ITS-4	reverse	TCCTCCGCTTATTGATATGC		
COI	bcdF01	forward	CATTTTCHACTAAYCATAARGATATTGG	Dabert et al. (2008)	Wełnicz et al. (2011)
	bcdR04	reverse	TATAAACYTCDGGATGNCCAAAAAA		

* – All PCR programmes are also provided in Stec et al. (2015).

DNA fragments were sequenced: four nuclear and one mitochondrial in the case of *E. insularis* sp. nov., four for *E. perarmatus* (excluding ITS-2, all will be presented in another contribution) and three for *P. mascarenensis* sp. nov. (excluding ITS-2 and COI). Both ITS-2 and COI are highly variable markers and are often difficult to amplify, as in these cases. All fragments were amplified and sequenced according to the protocols described in Stec et al. (2015). Primers and PCR programmes are presented in Table 2.

ITS-1 and ITS-2 sequences were used to reconstruct a concatenated Maximum Likelihood (ML) phylogeny for *E. insularis* sp. nov.; GenBank accession numbers for the sequences retrieved from GenBank are presented in the Suppl. material 6. Alignments were 741 bp (ITS-1) and 546 bp (ITS-2) long. SequenceMatrix was used for concatenation (Vaidya et al. 2011). ModelFinder (Kalyaanamoorthy et al. 2017) was used to choose the best-fit models: TIM3+F+G4 (ITS-1 partition) and TPM2+F+G4 (ITS-2 partition), chosen according to the Bayesian information criterion. W-IQ-TREE was used for ML reconstruction (Nguyen et al. 2015; Trifinopoulos et al. 2016). One thousand ultrafast bootstrap (UFBoot) replicates were applied to provide support values for branches (Hoang et al. 2018). Trees were rooted on *Diploechiniscus oihonnae* (Richters, 1903). The final consensus trees were visualised with FigTree ver. 1.4.3 (available at: http://tree.bio.ed.ac.uk/software/figtree/).

Results

Systematic account

Phylum: Tardigrada Doyère, 1840
Class: Heterotardigrada Marcus, 1927
Order: Echiniscoidea Richters, 1926
Family: Echiniscidae Thulin, 1928

Genus: *Bryodelphax* Thulin, 1928

Material. Single adult female on slide MU.001.01.

Remarks. A remarkably ornamented dorsum indicates that the individual found belongs to a new species. Its formal description is impossible with such scarce material.

Genus: *Echiniscus* C.A.S. Schultze, 1840

Echiniscus insularis sp. nov. Gąsiorek, Vončina & Kiosya

http://zoobank.org/1C027A99-2712-4825-A41A-3479E3768C3A

Figures 1–8, Tables 3–5

***Locus typicus* and type material.** ca. 20°22'S, 57°29'E, 580 m asl; Sophie Nature Walk, vicinity of Mare aux Vacoas (Plaines Wilhems, Mauritius, Mascarene Archipelago, Western Indian Ocean); mosses from tree trunks. Holotype (mature female on slide MU.002.04), allotype (mature male on slide MU.002.02), seven paratypic females, fourteen paratypic males, and five juveniles (slides MU.001.01–3, MU.002.01–6). One hologenophore on slide MU.001.24, and three hologenophores the slide MU.002.07. All deposited in the Department of Invertebrate Evolution.

Etymology. From Latin *insula* = island. The name refers to *locus typicus*. Adjective in the nominative singular.

Description. Mature females (i.e. from the third instar onwards; measurements in Table 3). Body small and plump (Figs 1, 3, 6A), yellow to orange, with minute red eyes absent after mounting. Ordinary primary and secondary (cephalic papillae) clavae of the *Echiniscus*-type; peribuccal cirri with well-developed cirrophores. Cirrus *A* very short (<25% of the body length), with cirrophore. Body appendage configuration A-(B)-C-C^d-D-D^d-E, with the majority of appendages developed as spicules, slightly longer spines can occur only in the positions C^d, D^d, and E (Figs 1, 3B, 6A). Asymmetries frequent, but only rarely are more appendages absent (Fig. 3A). Additionally, supernumerary spicules occur along the margins of all dorsal plates and sometimes on their surface (Fig. 6A), particularly frequent (up to five) along the caudal incisions (Fig. 6D, E). Spines and spicules are always smooth and simple, not ramified.

Dorsal plates strongly sclerotised and well-demarcated from each other, with the *spinulosus* type sculpturing, i.e. only pores are present (Figs 1, 3, 6A). Pores are densely arranged and may be of various size: from medium (Figs 1B, C, 3B, 6A) to large (Fig. 1A), even merging into groups of two/three pores (Fig. 3A). Dark endocuticular rings usually absent (Figs 1, 3B, 6A), or present, but only in the largest pores (Fig. 3A). Only in one female are pores absent and irregular dark epicuticular swellings are

Table 3. Measurements [in µm] of selected morphological structures of mature females of *Echiniscus insularis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD		Holotype	
		µm			*sp*			µm	*sp*	µm	*sp*	µm	*sp*
Body length	7	122	–	169	*466*	–	*591*	150	*514*	16	*41*	136	*529*
Scapular plate length	7	25.7	–	35.4		–		29.1	–	3.2	–	25.7	–
Head appendages lengths													
Cirrus *internus*	7	8.5	–	13.2	*32.4*	–	*40.9*	10.8	*37.1*	1.7	*3.4*	9.0	*35.0*
Cephalic papilla	7	5.0	–	7.0	*16.9*	–	*22.2*	5.9	*20.3*	0.6	*1.8*	5.7	*22.2*
Cirrus *externus*	7	10.9	–	15.9	*40.7*	–	*52.2*	13.5	*46.3*	1.6	*4.2*	12.9	*50.2*
Clava	7	3.6	–	5.5	*11.9*	–	*17.5*	4.4	*15.2*	0.6	*1.9*	4.5	*17.5*
Cirrus *A*	7	24.2	–	37.0	*86.1*	–	*106.2*	28.3	*96.9*	4.1	*7.0*	27.3	*106.2*
Cirrus *A*/Body length ratio	7	17%	–	22%		–		19%	–	2%	–	20%	–
Body appendages lengths													
Spine *B*	5	2.5	–	3.2	*8.8*	–	*10.8*	2.8	*9.8*	0.3	*0.7*	2.5	*9.7*
Spine *C*	7	2.0	–	5.2	*7.6*	–	*20.2*	4.0	*14.1*	1.3	*5.0*	5.2	*20.2*
Spine C^d	7	2.3	–	11.0	*8.8*	–	*39.9*	5.9	*20.6*	3.3	*11.6*	4.6	*17.9*
Spine *D*	6	2.5	–	4.0	*7.6*	–	*15.6*	3.1	*10.5*	0.6	*3.1*	4.0	*15.6*
Spine D^d	7	7.5	–	15.4	*22.6*	–	*53.3*	11.3	*39.5*	3.2	*12.4*	12.0	*46.7*
Spine *E*	5	2.2	–	9.6	*7.5*	–	*32.7*	6.5	*23.0*	2.7	*9.5*	6.0	*23.3*
Supernumerary spicules	24	0.7	–	4.6	*2.4*	–	*17.9*	–	–	–	–	–	–
Spine on leg I length	7	1.6	–	2.6	*5.8*	–	*7.5*	2.0	*6.7*	0.4	*0.7*	1.6	*6.2*
Papilla on leg IV length	7	2.8	–	3.5	*9.9*	–	*12.5*	3.1	*10.7*	0.2	*0.8*	3.2	*12.5*
Number of teeth on the collar	7	7	–	11		–		8.6	–	1.6	–	11	–
Claw 1 heights													
Branch	7	7.0	–	10.3	*26.2*	–	*30.7*	8.3	*28.5*	1.0	*1.8*	7.9	*30.7*
Spur	4	1.7	–	2.1	*5.8*	–	*8.2*	1.8	*6.6*	0.2	*1.1*	2.1	*8.2*
Spur/branch length ratio	4	20%	–	27%		–		23%	–	3%	–	27%	–
Claw 2 heights													
Branch	7	7.2	–	9.4	*24.5*	–	*30.8*	7.9	*27.2*	0.8	*2.5*	7.7	*30.0*
Spur	5	1.3	–	2.5	*4.4*	–	*7.1*	1.7	*5.5*	0.5	*1.1*	?	*?*
Spur/branch length ratio	5	18%	–	27%		–		21%	–	3%	–	?	–
Claw 3 heights													
Branch	7	6.9	–	9.9	*25.5*	–	*32.7*	8.1	*27.8*	1.0	*2.9*	8.4	*32.7*
Spur	5	1.3	–	2.2	*4.4*	–	*6.2*	1.7	*5.5*	0.4	*0.7*	?	*?*
Spur/branch length ratio	5	16%	–	24%		–		20%	–	3%	–	?	–
Claw 4 heights													
Branch	7	8.2	–	10.8	*28.8*	–	*35.8*	9.2	*31.7*	0.8	*2.1*	9.2	*35.8*
Spur	3	2.0	–	2.7	*6.8*	–	*8.7*	2.4	*7.7*	0.4	*0.9*	?	*?*
Spur/branch length ratio	3	21%	–	27%		–		24%	–	3%	–	?	–

developed, most visible on the scapular plate (Fig. 4A). The cephalic plate consists of two halves, with an anterior chalice-like incision (Figs 1A, 3A). The cervical (neck) plate is in the form of a narrow grey belt, weakly delineated anterior to the scapular plate (Figs 1A, 3). The scapular plate non-facetted, with the usual lateral sutures delineating small rectangular portions (Figs 1, 3). Three median plates: m1, m3 unipartite, the latter reduced to a narrow stripe, and m2 bipartite (Figs 3A, 6A); sculpture well-developed in all portions of the median plates with the exception of the anterior portion of m2, where it is reduced (Figs 1B, C, 3, 6A). Two pairs of large segmental plates, their narrower anterior portions with two thin belts devoid of sculpture (Figs 1B, 3, 6A) or with only one belt (Fig. 3B). The caudal (terminal) plate with evident incisions (Figs 1, 3) and may be facetted (Figs 3B, 6A).

Ventral cuticle smooth. Sexpartite gonopore located anteriorly of legs IV and a trilobed anus between legs IV. Pedal plates absent, but dim pulvini present (Figs 1B, 3A). Spine I thin and minute (Figs 1, 3A). Dentate collar IV composed of numerous acute teeth (Fig. 7D). Papilla on leg IV present (Figs 1, 3, 6A). Claws I–IV of similar heights. External claws on all legs smooth. Internal claws with proportionally large spurs positioned at ca. 1/4–1/3 of the claw height, spurs IV slightly heteromorphic since they are more divergent from the branches than spurs I–III (compare Fig. 7A–C, D).

Mature males (i.e. from the third instar onwards; measurements in Table 4). Body slender (Fig. 2). Only one male has rudimentary developed pores (Fig. 4B). Males have often comparatively better developed supernumerary spicules than females (Fig. 2B). Clavae enlarged, more prominent than in females (Figs 2, 6B). Subcephalic region with a pair of weakly developed oval swellings (probably rudimentary subcephalic plates). Gonopore circular, with a U-shaped slit; semicircular bulge resembling a genital plate with a cracked surface present anterior to the gonopore (Fig. 6C).

Juveniles (i.e. from the second instar onwards; measurements in Table 5). No morphometric gap between adults and juveniles (likely a result of general miniaturisation of the species). Qualitatively similar to adults (Fig. 5). Gonopore absent.

Larvae. Unknown.

Eggs. One egg per exuviae was found in few examined exuviae.

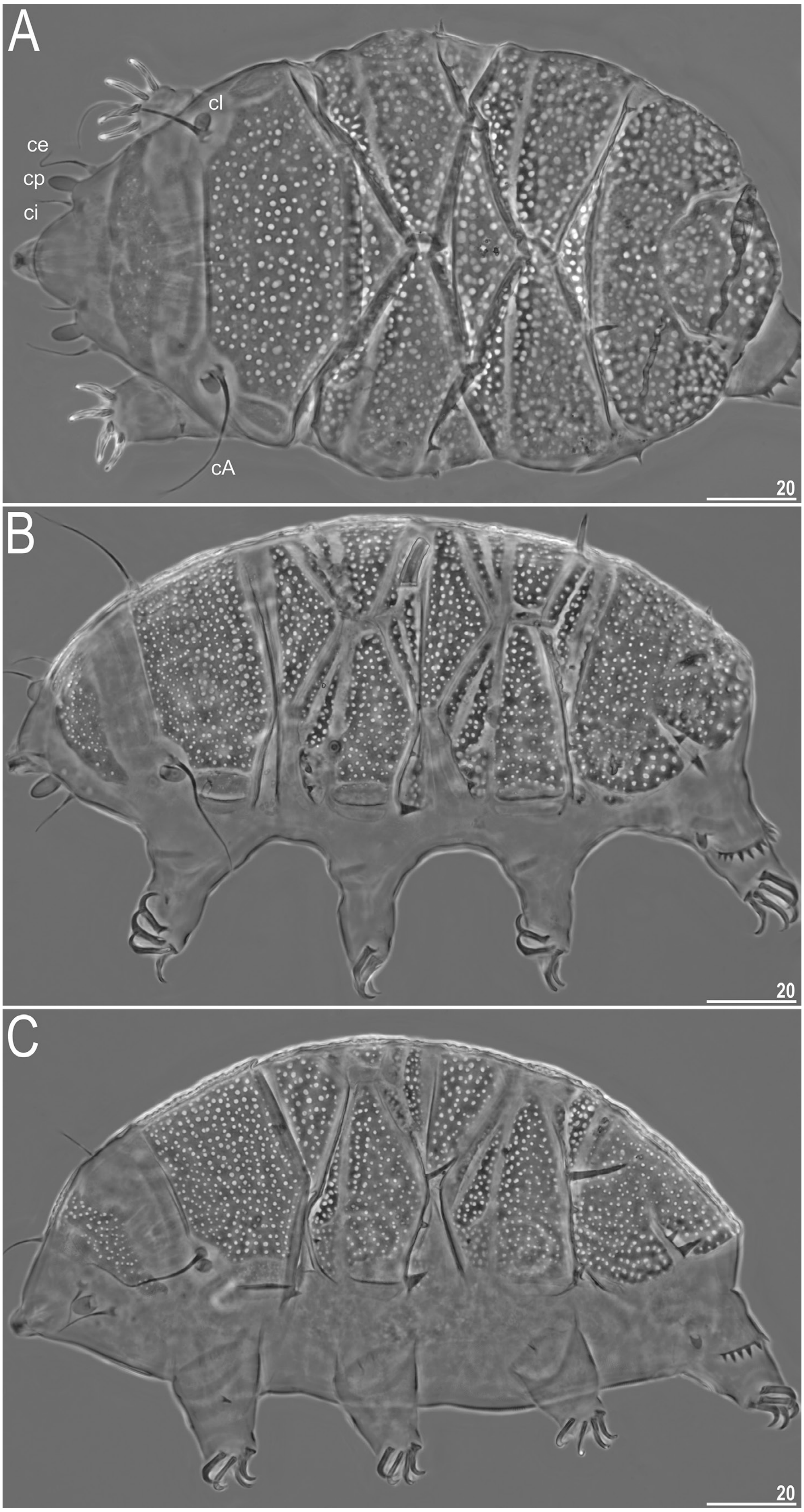

Figure 1. Habitus of females of *Echiniscus insularis* sp. nov. (PCM): **A** dorsal view (cA – cirrus *A*, ce – *cirrus externus*, ci – *cirrus internus*, cl – (primary) clava, cp – cephalic papilla), **B** dorsolateral view, **C** lateral view. Note irregularly distributed spicules along margins of the dorsal plates. Scale bars in μm.

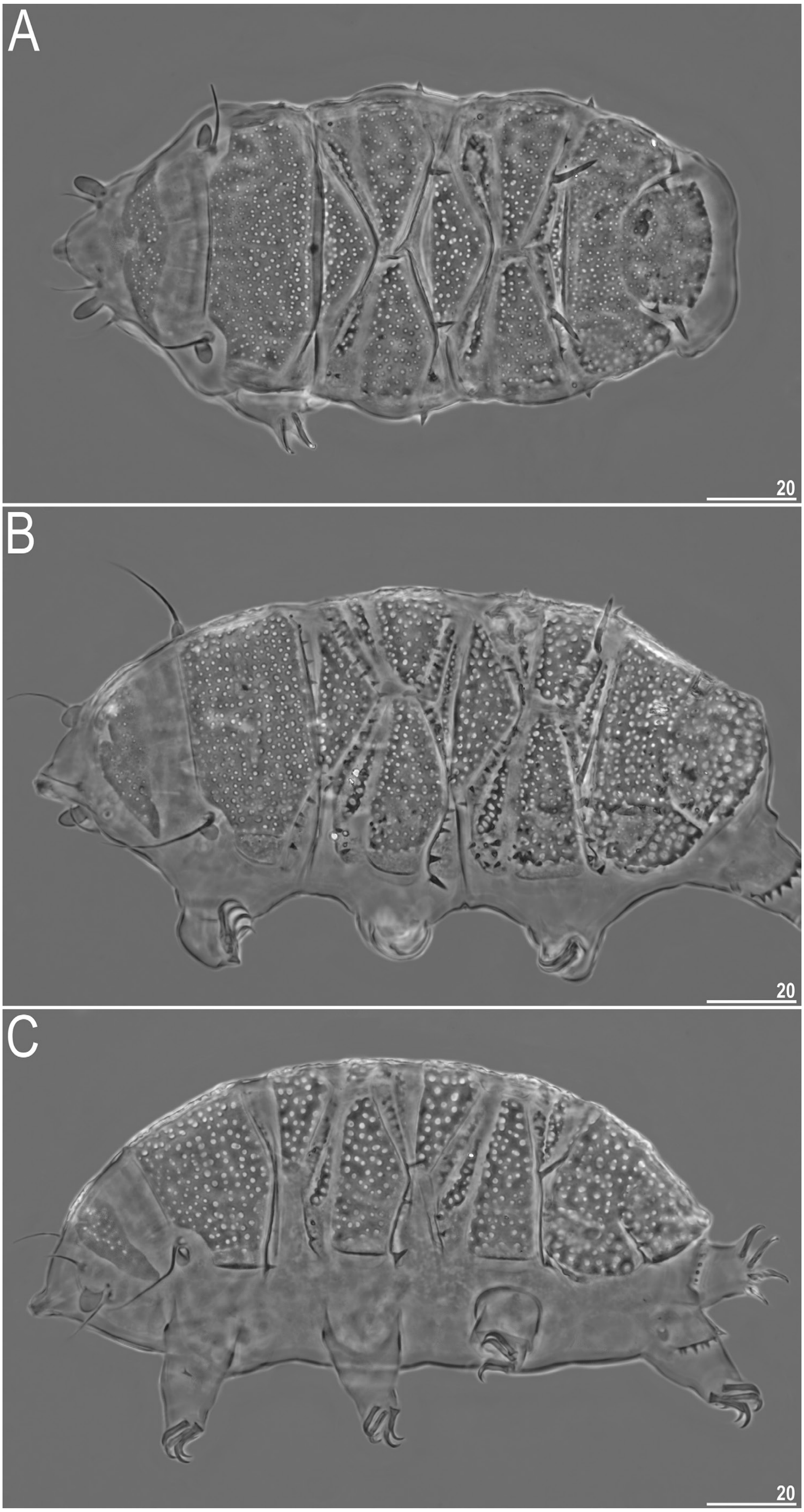

Figure 2. Habitus of males of *Echiniscus insularis* sp. nov. (PCM): **A** dorsal view, **B** dorsolateral view, **C** lateral view. Note differences between the density of supernumerary spicules at margins of the dorsal plates. Scale bars in μm.

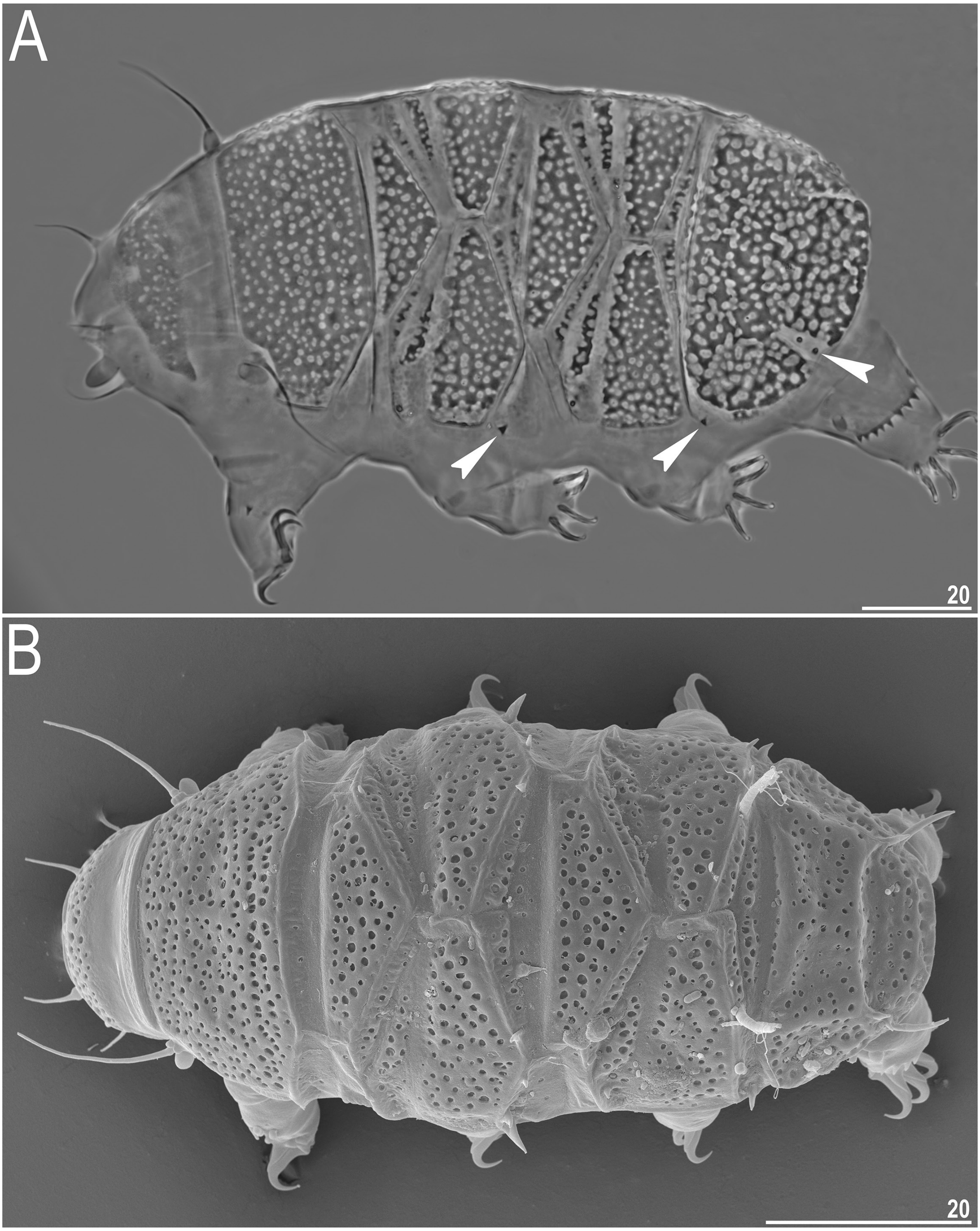

Figure 3. Habitus of females of *Echiniscus insularis* sp. nov.: **A** specimen with appendages (arrowheads) greatly reduced in number and aberrantly large, merging pores (PCM, dorsolateral view), **B** typical female with ordinary set of appendages (SEM, dorsal view). Scale bars in μm.

DNA sequences and phylogenetic position. Two haplotypes in all markers were found, corresponding with the populations MU.001 and MU.002: 18S rRNA (MW180887, MW180888), 28S rRNA (MW180879, MW180880), ITS-1 (MW180910, MW180911), ITS-2 (MW180898, MW180899), and in COI (MW178242, MW178243). *p*-distance in COI between the two populations is 4.9%. *Echiniscus insularis* sp. nov. belongs

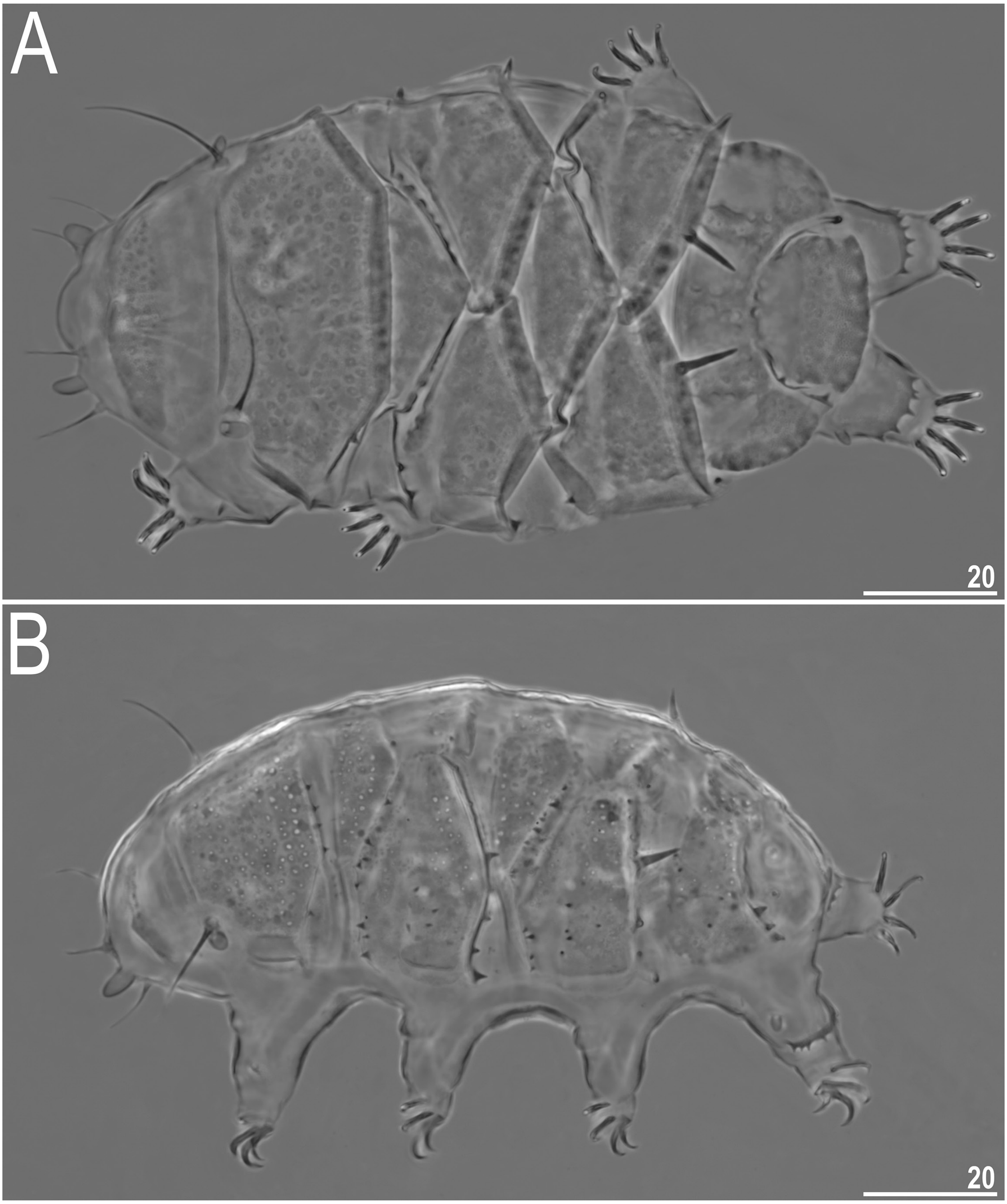

Figure 4. Habitus of *Echiniscus insularis* sp. nov. – individuals with aberrantly developed dorsal sculpturing (PCM): **A** female, dorsal view, **B** male, dorsolateral view. Note differences in the development of appendages. Scale bars in μm.

in the *spinulosus* complex, being a sister species to the clade composed of *E. manuelae* da Cunha & do Nascimento Ribeiro, 1962 + *E. tristis* Gąsiorek & Kristensen, 2018 (Fig. 8).

Remarks. The species is easily recognisable because of the additional supernumerary dorsal spicules along margins of all plates and sometimes on the plates, making it an unusual member of the *spinulosus* group and of the entire genus. Besides, it is one of the smallest representatives of *Echiniscus* with the average adult body length at ca. 150 μm, whereas adults of *Echiniscus* spp. usually reach 200–250 μm at least. There is one species

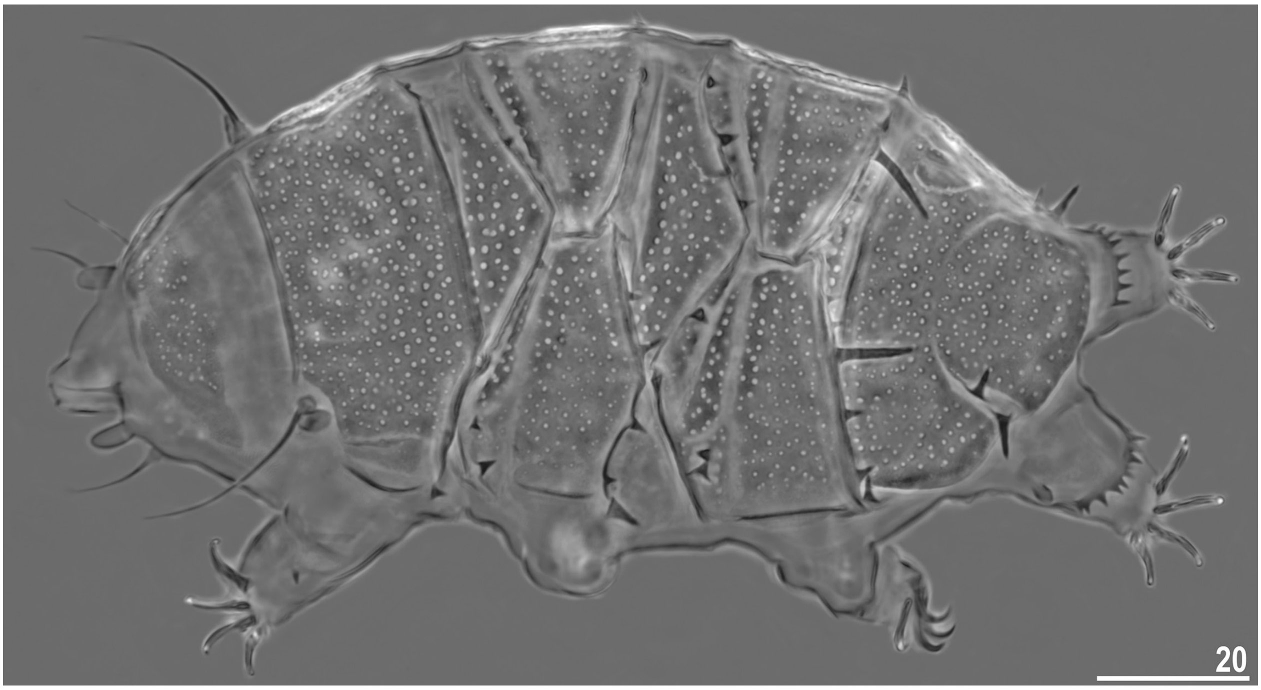

Figure 5. Habitus of juvenile of *Echiniscus insularis* sp. nov. with fully developed appendages (PCM, dorsolateral view). Scale bar in µm.

Table 4. Measurements [in µm] of selected morphological structures of mature males (one hologenophore included) of *Echiniscus insularis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD		Allotype	
		µm			*sp*			µm	*sp*	µm	*sp*	µm	*sp*
Body length	15	113	–	167	*500*	–	*596*	145	*548*	15	*28*	167	*582*
Scapular plate length	15	22.2	–	28.8		–		26.4	–	1.8	–	28.7	–
Head appendages lengths													
Cirrus *internus*	14	5.6	–	13.8	*23.0*	–	*49.6*	10.0	*37.5*	2.2	*7.1*	9.4	*32.8*
Cephalic papilla	15	4.5	–	7.2	*18.5*	–	*27.8*	6.4	*24.1*	0.7	*2.6*	7.2	*25.1*
Cirrus *externus*	15	7.9	–	18.0	*32.5*	–	*64.7*	13.7	*51.6*	2.7	*8.2*	15.6	*54.4*
Clava	15	3.2	–	6.1	*14.4*	–	*23.1*	4.8	*18.0*	0.8	*2.7*	5.8	*20.2*
Cirrus *A*	15	17.2	–	29.9	*77.5*	–	*112.7*	24.1	*90.9*	3.6	*9.5*	29.9	*104.2*
Cirrus *A*/Body length ratio	15	15%	–	20%		–		17%	–	1%	–	18%	–
Body appendages lengths													
Spine *B*	6	1.6	–	2.9	*6.0*	–	*11.1*	2.4	*8.7*	0.5	*1.8*	?	*?*
Spine *C*	15	2.2	–	5.0	*8.3*	–	*17.4*	3.7	*13.9*	0.7	*2.4*	5.0	*17.4*
Spine *C*^{d}	5	2.4	–	8.0	*10.8*	–	*32.9*	5.4	*21.2*	2.0	*7.9*	?	*?*
Spine *D*	15	1.9	–	3.5	*6.8*	–	*13.3*	2.6	*9.8*	0.5	*2.1*	2.7	*9.4*
Spine *D*^{d}	14	3.2	–	13.2	*13.1*	–	*49.2*	10.1	*38.3*	2.7	*9.7*	11.4	*39.7*
Spine *E*	13	3.9	–	7.7	*14.8*	–	*28.2*	5.9	*21.9*	1.3	*4.9*	5.7	*19.9*
Supernumerary spicules	32	0.6	–	3.0	*2.1*	–	*11.5*	–	–	–	–	–	–
Spine on leg I length	15	1.2	–	2.4	*4.9*	–	*9.2*	1.8	*6.9*	0.4	*1.3*	1.9	*6.6*
Papilla on leg IV length	15	2.3	–	4.0	*9.5*	–	*13.9*	3.1	*11.8*	0.4	*1.4*	4.0	*13.9*
Number of teeth on the collar	15	7	–	11		–		9.2	–	1.3	–	11	–
Claw 1 heights													
Branch	13	5.7	–	9.7	*23.5*	–	*34.2*	8.3	*31.2*	1.2	*2.9*	9.7	*33.8*
Spur	9	1.5	–	2.3	*6.2*	–	*8.7*	1.8	*6.9*	0.2	*0.8*	2.1	*7.3*
Spur/branch length ratio	9	20%	–	26%		–		22%	–	2%	–	22%	–
Claw 2 heights													
Branch	14	5.7	–	9.2	*23.5*	–	*33.1*	7.8	*29.5*	1.1	*2.7*	8.8	*30.7*
Spur	10	1.3	–	2.4	*5.2*	–	*9.4*	1.7	*6.5*	0.3	*1.3*	1.6	*5.6*
Spur/branch length ratio	10	17%	–	29%		–		21%	–	4%	–	18%	–
Claw 3 heights													
Branch	15	5.0	–	9.3	*20.6*	–	*33.7*	7.9	*29.9*	1.2	*3.1*	9.1	*31.7*
Spur	7	1.2	–	2.0	*4.9*	–	*7.8*	1.7	*6.5*	0.3	*0.9*	?	*?*
Spur/branch length ratio	7	17%	–	25%		–		22%	–	2%	–	?	–
Claw 4 heights													
Branch	15	6.4	–	11.2	*26.3*	–	*42.4*	9.4	*35.4*	1.4	*4.2*	11.2	*39.0*
Spur	8	1.5	–	2.6	*6.2*	–	*10.2*	2.3	*8.7*	0.4	*1.2*	?	*?*
Spur/branch length ratio	8	22%	–	28%		–		25%	–	2%	–	?	–

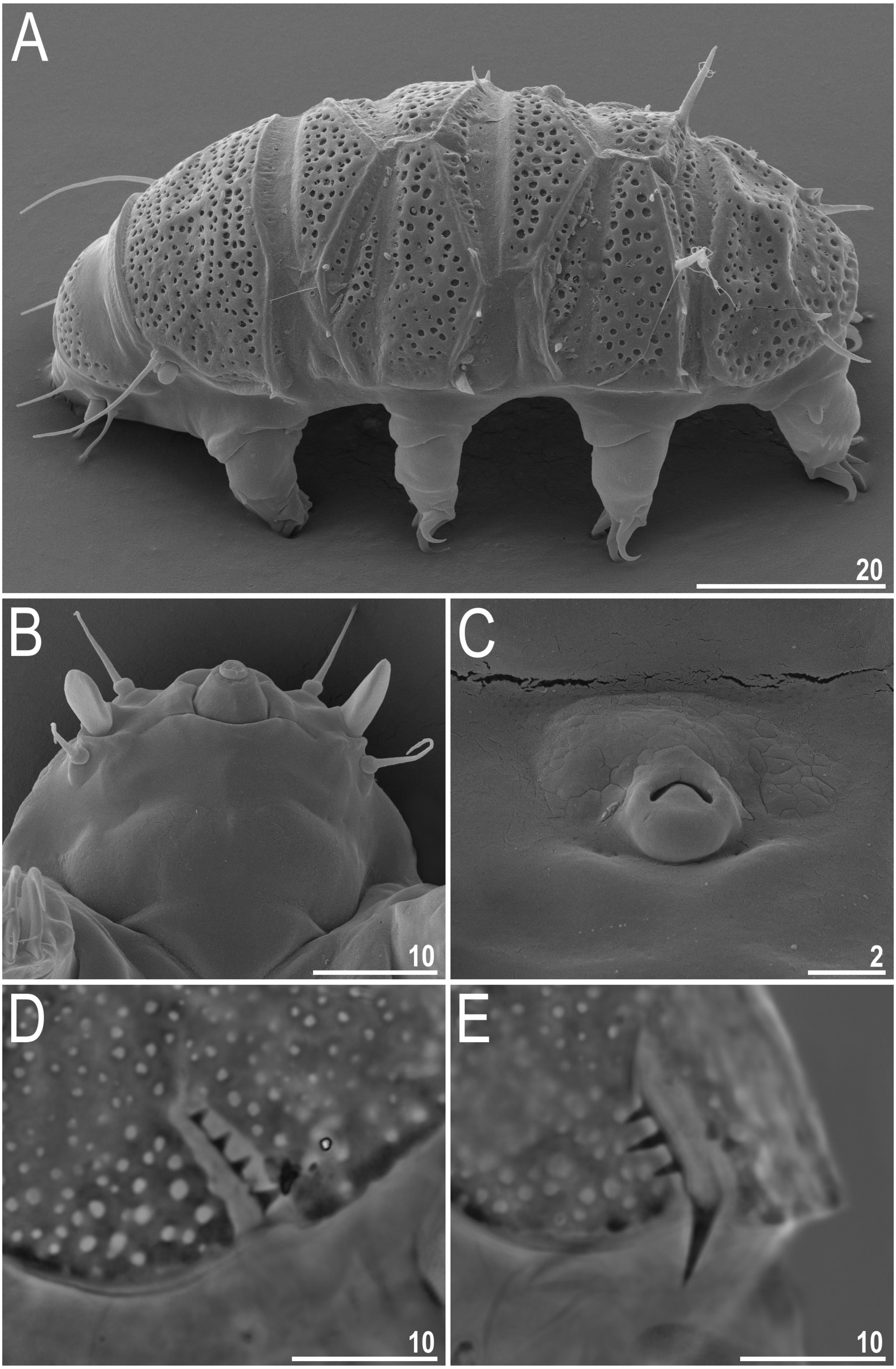

Figure 6. Morphological details of *Echiniscus insularis* sp. nov.: **A** female in lateral view (SEM), **B** male cephalic appendages (SEM), **C** male gonopore (SEM), **D, E** appendages along the caudal incision (PCM). Scale bars in µm.

Table 5. Measurements [in µm] of selected morphological structures of juveniles of *Echiniscus insularis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range µm			Range *sp*			Mean µm	Mean *sp*	SD µm	SD *sp*
Body length	5	100	–	140	*422*	–	*625*	121	*507*	18	*75*
Scapular plate length	5	18.4	–	29.4		–		24.2	–	4.3	–
Head appendages lengths											
Cirrus *internus*	5	5.3	–	9.5	*22.4*	–	*34.7*	7.4	*30.6*	1.6	*5.1*
Cephalic papilla	5	3.5	–	5.6	*16.8*	–	*21.5*	4.6	*18.9*	0.8	*1.7*
Cirrus *externus*	5	6.6	–	13.0	*35.9*	–	*50.6*	10.8	*44.0*	2.8	*5.6*
Clava	5	3.0	–	4.7	*13.5*	–	*18.5*	3.8	*15.8*	0.7	*2.0*
Cirrus *A*	5	15.3	–	27.3	*83.2*	–	*101.3*	22.4	*92.0*	5.2	*8.3*
Cirrus *A*/Body length ratio	5	13%	–	24%		–		19%	–	4%	–
Body appendages lengths											
Spine *B*	1	2.2	–	2.2	*9.3*	–	*9.3*	2.2	*9.3*	?	*?*
Spine *C*	5	2.2	–	4.2	*12.0*	–	*18.9*	3.7	*15.4*	0.9	*2.9*
Spine C^d	4	2.1	–	8.4	*7.1*	–	*35.4*	5.6	*22.3*	2.9	*11.9*
Spine *D*	5	1.3	–	3.7	*7.1*	–	*15.6*	2.7	*11.0*	0.9	*3.3*
Spine D^d	5	6.7	–	13.0	*36.4*	–	*54.9*	10.6	*43.8*	2.4	*7.3*
Spine *E*	4	4.1	–	7.3	*22.1*	–	*30.8*	5.8	*24.9*	1.5	*4.1*
Supernumerary spicules	18	1.1	–	3.0	*5.0*	–	*12.7*	–	–	–	–
Spine on leg I length	4	1.1	–	2.6	*5.0*	–	*8.8*	1.8	*7.1*	0.8	*2.0*
Papilla on leg IV length	5	2.1	–	2.9	*9.9*	–	*12.2*	2.6	*10.7*	0.4	*1.1*
Number of teeth on the collar	5	6	–	10		–		7.8	–	1.5	–
Claw 1 heights											
Branch	5	5.6	–	8.1	*26.1*	–	*32.1*	7.0	*29.1*	1.2	*2.4*
Spur	3	1.0	–	2.1	*4.5*	–	*7.1*	1.5	*6.2*	0.6	*1.5*
Spur/branch length ratio	3	17%	–	26%		–		22%	–	5%	–
Claw 2 heights											
Branch	5	4.9	–	7.7	*25.2*	–	*28.7*	6.5	*26.8*	1.2	*1.5*
Spur	3	1.0	–	1.7	*5.4*	–	*5.8*	1.3	*5.5*	0.4	*0.2*
Spur/branch length ratio	3	20%	–	23%		–		22%	–	1%	–
Claw 3 heights											
Branch	5	5.3	–	7.3	*24.8*	–	*28.8*	6.4	*26.6*	1.0	*1.8*
Spur	3	1.0	–	1.6	*5.4*	–	*5.9*	1.3	*5.6*	0.3	*0.2*
Spur/branch length ratio	3	19%	–	24%		–		21%	–	2%	–
Claw 4 heights											
Branch	4	6.3	–	8.6	*27.6*	–	*34.2*	7.4	*30.5*	1.2	*3.0*
Spur	1	2.0	–	2.0	*6.8*	–	*6.8*	2.0	*6.8*	?	*?*
Spur/branch length ratio	1	25%	–	25%		–		25%	–	?	–

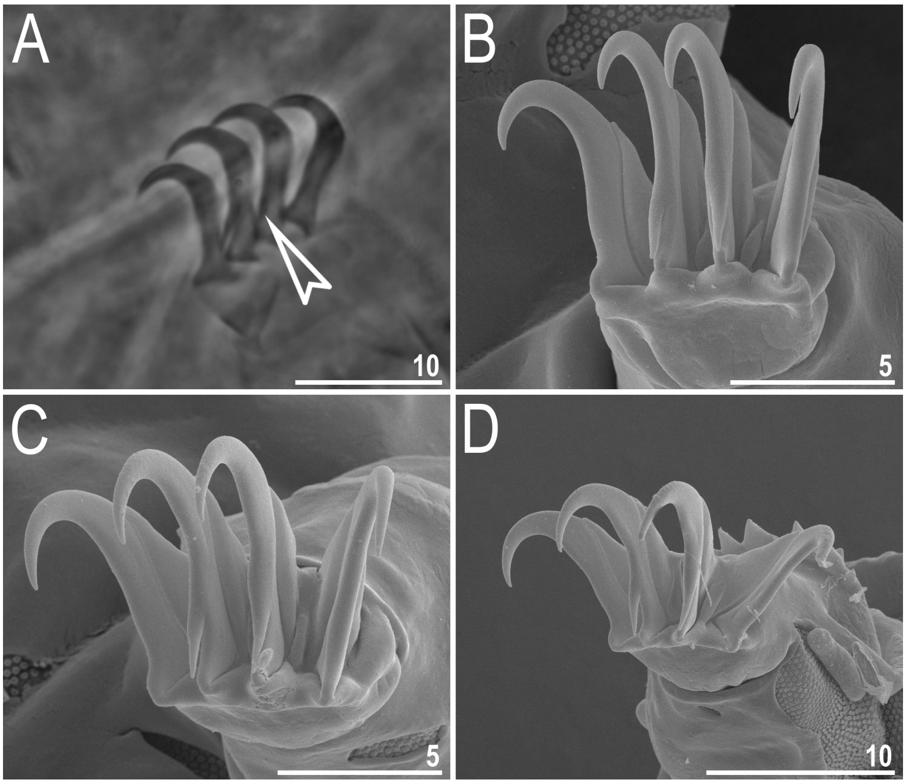

Figure 7. Claws of *Echiniscus insularis* sp. nov.: **A** claws I (PCM, empty arrowhead indicates the asymmetric lack of internal spur), **B** claws II (SEM), **C** claws III (SEM), **D** claws IV with dentate collar (SEM). Scale bars in µm.

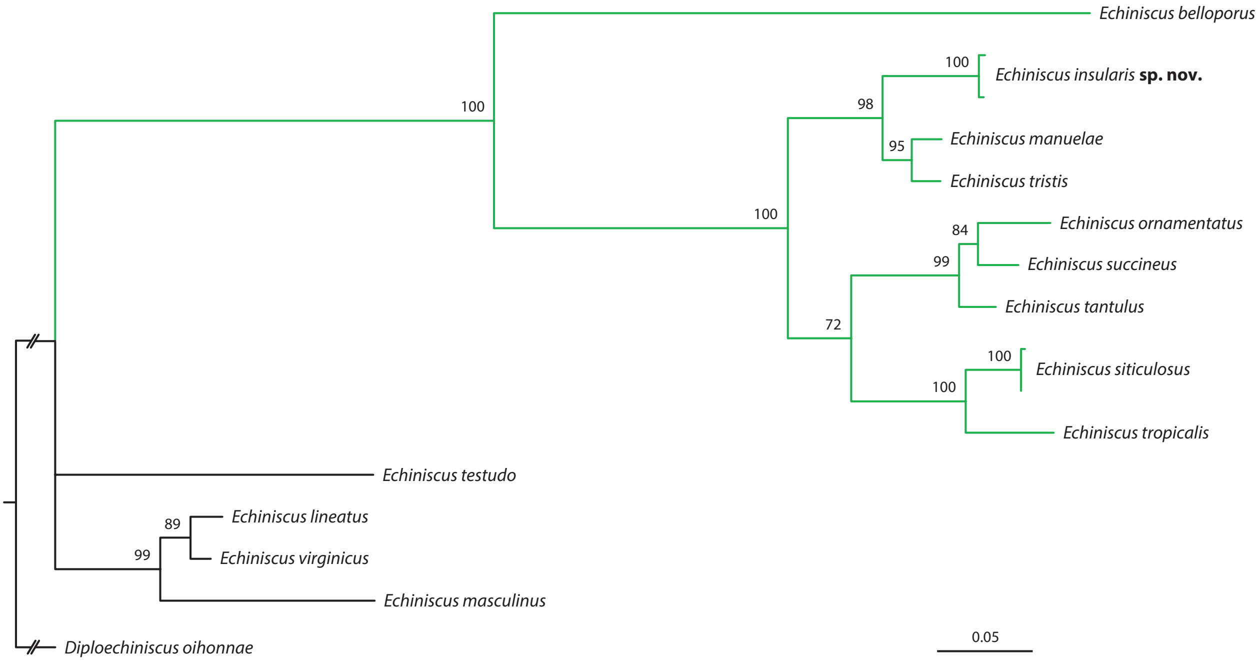

Figure 8. Phylogenetic position of *Echiniscus insularis* sp. nov. on the Maximum Likelihood consensus phylogenetic tree; the *E. spinulosus* complex is marked in green and *Diploechiniscus oihonnae* was used as an outgroup. ML bootstrap values are presented above the branches.

resembling specimens of *E. insularis* sp. nov. with a lower number of spicules – *E. tropicalis* Binda & Pilato, 1995 described from the Seychelles. For the purpose of the comparison *E. insularis* sp. nov. – *E. tropicalis*, we present updated description of the latter species below.

Due to the fact that *E. manuelae* and *E. tristis* currently emerge as species closest phylogenetically to *E. insularis* sp. nov., we compare them with the new species accordingly:

- *E. manuelae* has larger and more sparsely distributed pores in dorsal plates (see fig. 3 in da Cunha & do Nascimento Ribeiro (1962) and fig. 5 in Gąsiorek and Kristensen (2018)), and appendages $C^d + D^d$ are long and serrated (smooth and short in *E. insularis* sp. nov.);
- *E. tristis* is a larger species (adult females ≥ 180 µm in *E. tristis* vs < 170 µm in *E. insularis* sp. nov.) and has larger claw spurs that are more divergent from branches than in *E. insularis* sp. nov.

Echiniscus tropicalis Binda & Pilato, 1995

Figures 8–11, Tables 6–8

Material. Together 402 adult females, 17 juveniles and 10 larvae mounted on slides.

Description. Mature females (i.e. from the third instar onwards; measurements in Table 6). Body small and plump (Figs 9A, 11A), yellow to orange, with minute red eyes absent after mounting. Ordinary primary and secondary (cephalic papillae) clavae of the *Echiniscus*-type; peribuccal cirri with well-developed cirrophores. Cirrus *A* very short (<25% of the body length), with cirrophore. Body appendage configuration *A-B-C-*C^d*-D-*D^d*-E*, with all appendages developed as spines or spicules, which are smooth or only sometimes spines *E* are serrated (Figs 9A, 10, 11A). Asymmetries frequent, especially in the lateral positions.

Dorsal plates strongly sclerotised and well-demarcated from each other, with the *spinulosus* type sculpturing, i.e. only pores are present (Figs 9A, 10, 11). Pores are densely arranged and rather of uniform size. Dark endocuticular rings absent (Figs 10, 11B, C). The cephalic plate consists of two halves, with an anterior chalice-like incision. The cervical (neck) plate is in the form of a narrow grey belt, weakly delineated anterior to the scapular plate (Fig. 10). The scapular plate non-facetted, with the usual lateral sutures delineating small rectangular portions (Figs 9A, 10). Three median plates: m1, m3 unipartite, the latter reduced to a narrow stripe; m2 bipartite (Figs 9A, 10, 11). Two pairs of large segmental plates, their narrower anterior portions with two thin belts devoid of sculpture (Fig. 10). The caudal (terminal) plate with evident incisions (Figs 9A, 10) and may be facetted (Fig. 11A).

Ventral cuticle smooth or with densely arranged endocuticular pillars. Sexpartite gonopore located anteriorly of legs IV and a trilobed anus between legs IV. Pedal plates and pulvini present (Fig. 9A). Spine I thin and minute (Fig. 9A). Dentate collar IV composed of numerous acute teeth (Figs 9A, 11A). Papilla on leg IV present (Fig. 9A). Claws IV slightly higher than claws I–III. External claws on all legs smooth. Internal claws with heteromorphic spurs positioned at ca. 1/4–1/3 of the claw height.

Mature males. Absent.

Juveniles (i.e. from the second instar onwards; measurements in Table 7). No morphometric gap or qualitative differences between adult and juvenile females found. Gonopore absent.

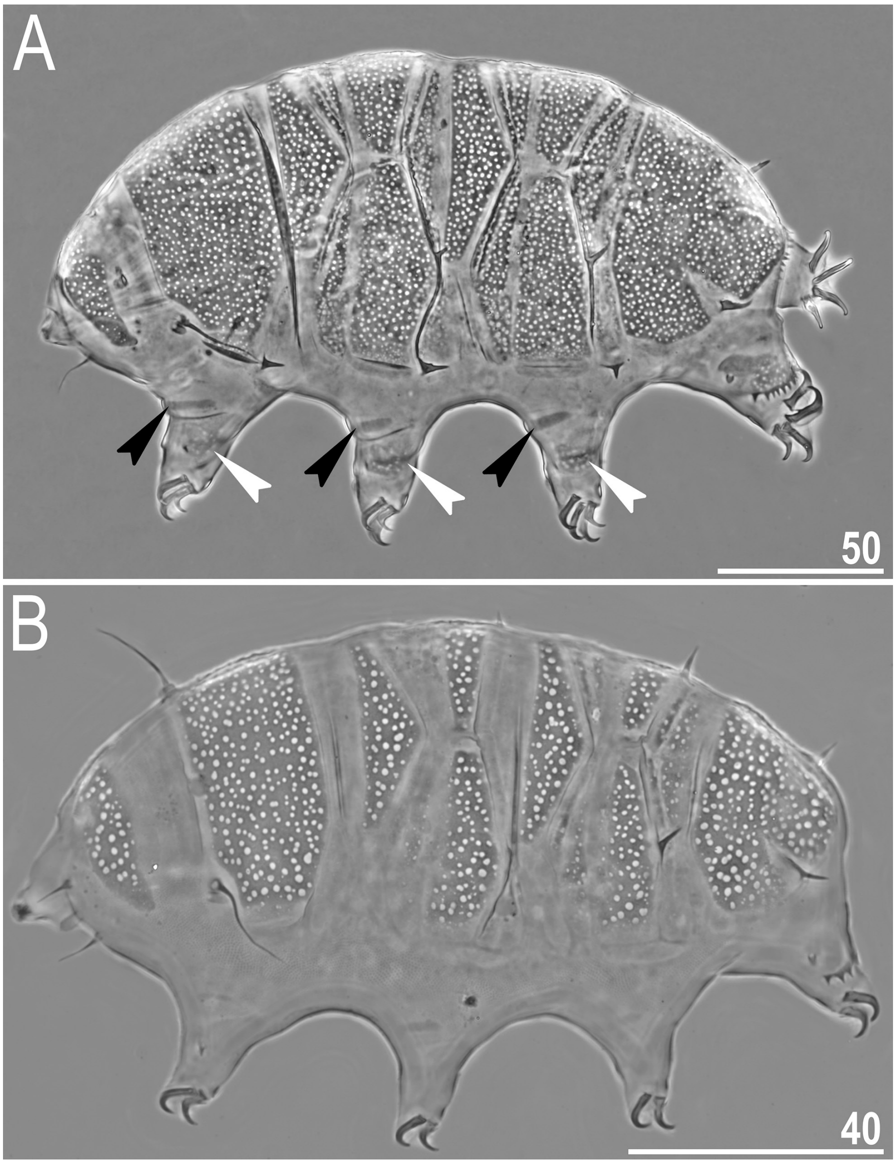

Figure 9. Habitus of *Echiniscus tropicalis* in dorsolateral view (PCM): **A** adult female (black arrowheads indicate pulvini, whereas white arrowheads – pedal plates), **B** larva. Scale bars in μm.

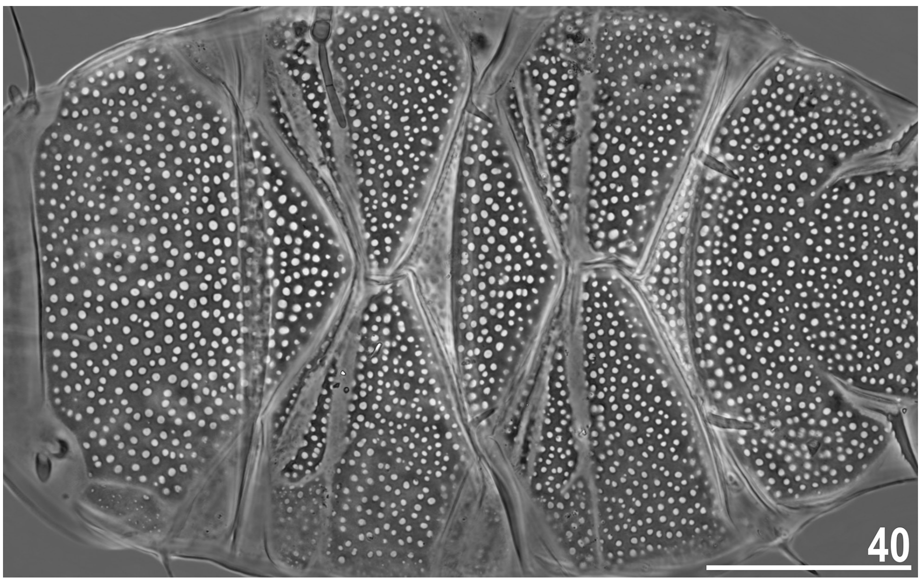

Figure 10. Dorsal plate sculpturing of *Echiniscus tropicalis* in close-up (PCM). Scale bar in μm.

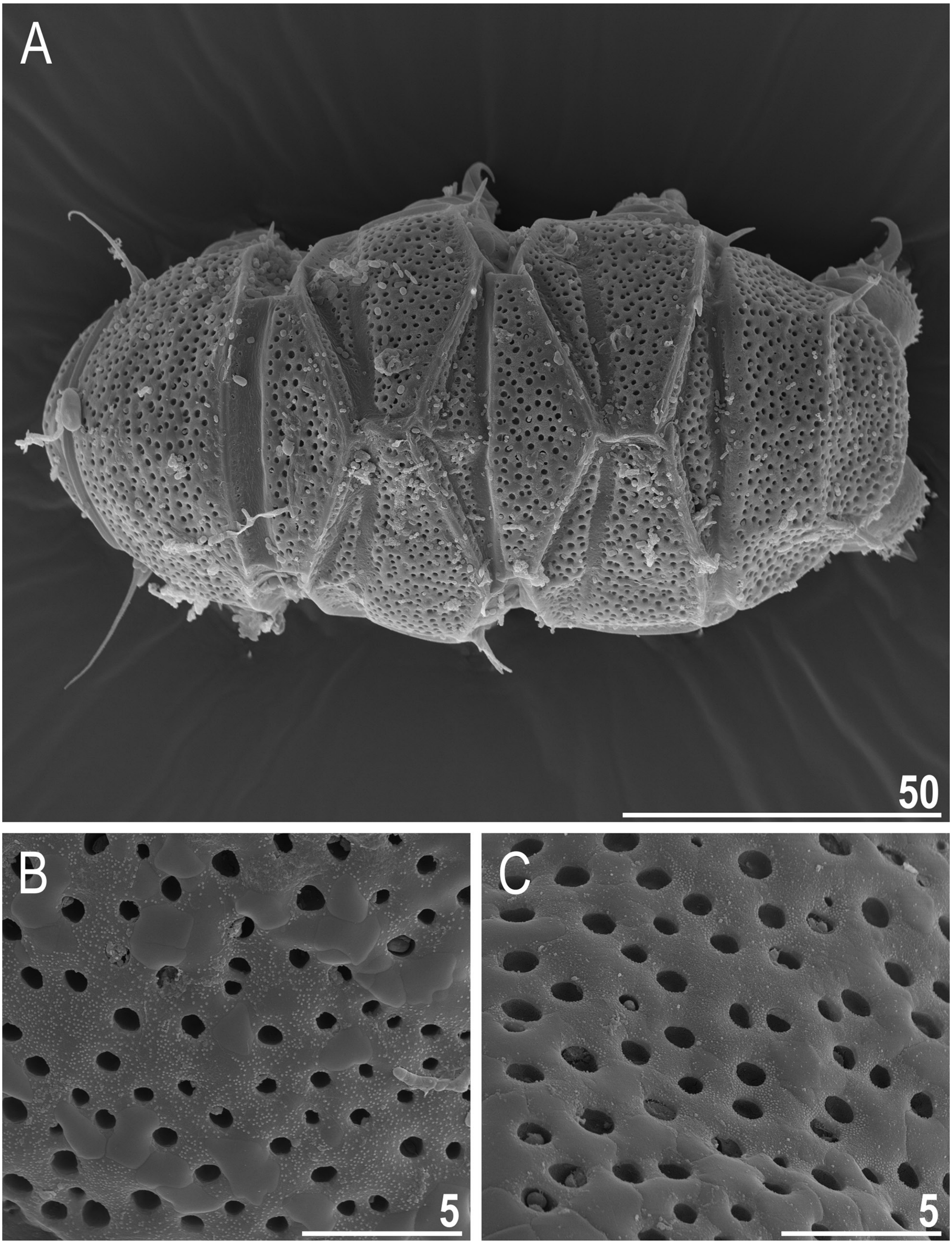

Figure 11. Dorsal plate sculpturing of *Echiniscus tropicalis* (SEM): **A** adult female in dorsal view, **B, C** pores in close-up. Scale bars in μm.

Larvae (i.e. the first instar; measurements in Table 8). Clear morphometric gap between juveniles and larvae exists (compare Tables 7, 8). Body appendage configuration *A*-C^d-D^d-*E* (Fig. 9B). Anterior portions of paired segmental plates weakly sclerotised. Gonopore and anus absent.

Table 6. Measurements [in µm] of selected morphological structures of mature females of *Echiniscus tropicalis* (pooled data from the populations ID.032, ID.939 and SG.001) mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD	
		µm			*sp*			µm	*sp*	µm	*sp*
Body length	26	137	–	223	*384*	–	*513*	194	*476*	20	*31*
Scapular plate length	26	33.6	–	45.2		–		40.9	–	3.3	–
Head appendages lengths											
Cirrus *internus*	24	10.0	–	14.5	*22.2*	–	*36.0*	12.3	*30.0*	1.3	*3.1*
Cephalic papilla	26	5.0	–	7.7	*12.6*	–	*18.8*	6.1	*14.9*	0.5	*1.5*
Cirrus *externus*	25	11.2	–	18.2	*27.5*	–	*41.7*	14.8	*36.4*	1.9	*3.2*
Clava	25	4.2	–	6.4	*9.3*	–	*16.3*	5.0	*12.2*	0.6	*1.6*
Cirrus *A*	25	17.9	–	33.8	*41.1*	–	*79.3*	27.8	*68.1*	3.7	*8.2*
Cirrus *A*/Body length ratio	25	9%	–	18%		–		14%	–	2%	–
Body appendages lengths											
Spine *B*	24	4.3	–	12.7	*9.9*	–	*29.4*	8.5	*20.8*	2.4	*5.5*
Spine *C*	26	6.5	–	14.7	*14.9*	–	*35.0*	11.1	*27.1*	2.3	*5.1*
Spine C^d	26	3.0	–	10.8	*7.7*	–	*25.0*	6.7	*16.4*	1.7	*4.0*
Spine *D*	22	5.7	–	13.8	*13.5*	–	*31.3*	10.0	*24.3*	2.4	*5.3*
Spine D^d	25	4.3	–	14.9	*9.9*	–	*35.0*	10.2	*25.1*	2.6	*6.3*
Spine *E*	26	7.1	–	15.8	*16.3*	–	*38.0*	12.1	*29.6*	2.3	*5.5*
Spine on leg I length	26	1.9	–	3.7	*4.2*	–	*8.8*	2.6	*6.4*	0.5	*1.1*
Papilla on leg IV length	25	2.8	–	4.3	*6.7*	–	*10.9*	3.4	*8.3*	0.3	*1.0*
Number of teeth on the collar	25	8	–	17		–		12.3	–	2.3	–
Claw 1 heights											
Branch	25	9.6	–	12.4	*24.7*	–	*30.4*	10.9	*26.8*	0.8	*1.5*
Spur	23	1.5	–	2.6	*4.0*	–	*6.0*	2.0	*4.9*	0.3	*0.5*
Spur/branch length ratio	23	15%	–	21%		–		18%	–	2%	–
Claw 2 heights											
Branch	26	9.2	–	12.0	*22.9*	–	*28.6*	10.4	*25.6*	0.8	*1.5*
Spur	25	1.6	–	2.7	*3.9*	–	*6.2*	1.9	*4.7*	0.3	*0.5*
Spur/branch length ratio	25	16%	–	23%		–		19%	–	2%	–
Claw 3 heights											
Branch	25	8.7	–	12.3	*23.2*	–	*28.6*	10.4	*25.6*	0.8	*1.5*
Spur	23	1.5	–	2.7	*3.8*	–	*6.2*	1.9	*4.7*	0.3	*0.6*
Spur/branch length ratio	23	15%	–	23%		–		18%	–	2%	–
Claw 4 heights											
Branch	26	10.7	–	14.8	*25.9*	–	*34.9*	12.6	*30.8*	1.1	*1.9*
Spur	18	2.0	–	3.1	*5.3*	–	*7.1*	2.4	*6.0*	0.2	*0.5*
Spur/branch length ratio	18	18%	–	24%		–		20%	–	2%	–

Eggs. One egg per exuviae was found in few examined exuviae.

DNA sequences and phylogenetic position. Two haplotypes in all markers were found, corresponding with the populations ID.032 and ID.939: 18S rRNA (MW327546, MW327547), 28S rRNA (MW327542, MW327543), ITS-2 (MW327549, MW327550), with the exception of ITS-1, characterised by one haplotype (MW327551, MW327552). The sister species of *E. tropicalis* within the *spinulosus* complex is *E. siticulosus* (Fig. 8).

Phenotypic differential diagnosis. *Echiniscus tropicalis* was originally described based on two adult females (Binda and Pilato 1995). We compared the newly found Southeast Asian specimens with the microphotographs of the holotype that confirmed our suspicions after reading the description, i.e. the lack of sound morphological discrepancies between the type material from the Seychelles and abundant material from the Malay Archipelago and the Malay Peninsula. The only difference is the serration of spines *E* that may be well-developed in Asian populations (Fig. 10), whereas this trait was not reported by Binda and Pilato (1995). The original description mentions "primary and secondary points" in the paratype = a potential ramification. As there is a considerable intrapopulation variability regarding this trait, the Seychellois and Asian populations should be ascertained as conspecific unless DNA data from the Seychelles reject this hypothesis.

There is a plethora of differences between adult females of *E. insularis* sp. nov. and *E. tropicalis* after the description of the latter was supplemented with new data:

- the presence of supernumerary spicules along the margins of dorsal plates and in the caudal incisions (present in *E. insularis* sp. nov. vs absent in *E. tropicalis*);
- the presence of pedal plates (absent in *E. insularis* sp. nov. vs present in *E. tropicalis*);
- the relative length of cirrus *A* (*86.1–106.2* in *E. insularis* sp. nov. vs *41.1–79.3* in *E. tropicalis*);
- the absolute lengths of lateral spines *B*–*D* (*B* 2.5–3.2 µm, *C* 2.0–5.2 µm, *D* 2.5–4.0 µm in *E. insularis*

Table 7. Measurements [in µm] of selected morphological structures of juveniles of *Echiniscus tropicalis* (population ID.939) mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD	
		µm			*sp*			µm	*sp*	µm	*sp*
Body length	10	143	–	169	444	–	506	158	475	9	19
Scapular plate length	10	29.9	–	35.4		–		33.2	–	1.8	–
Head appendages lengths											
Cirrus *internus*	9	7.6	–	9.6	22.4	–	29.0	8.7	25.8	0.8	2.2
Cephalic papilla	9	4.6	–	5.5	13.3	–	17.5	5.1	15.5	0.3	1.2
Cirrus *externus*	10	10.2	–	13.7	34.1	–	41.8	12.1	36.4	0.9	2.3
Clava	10	3.6	–	4.5	11.2	–	12.9	4.0	12.2	0.3	0.6
Cirrus *A*	9	22.2	–	25.6	63.3	–	78.0	23.4	71.1	1.3	5.3
Cirrus *A*/Body length ratio	9	13%	–	16%		–		15%	–	1%	–
Body appendages lengths											
Spine *B*	9	3.5	–	7.4	10.4	–	22.8	5.3	15.7	1.5	4.4
Spine *C*	10	6.5	–	10.5	19.9	–	33.4	8.7	26.3	1.5	4.4
Spine C^{d}	10	4.6	–	7.9	14.1	–	24.4	6.5	19.6	1.0	2.9
Spine *D*	7	2.1	–	8.8	6.4	–	26.1	5.3	15.9	2.5	7.8
Spine D^{d}	10	10.1	–	14.3	28.9	–	42.2	11.4	34.2	1.3	3.8
Spine *E*	10	7.8	–	13.0	23.9	–	40.1	11.2	33.8	1.5	4.3
Spine on leg I length	10	1.8	–	2.6	5.3	–	7.9	2.1	6.2	0.2	0.8
Papilla on leg IV length	10	2.4	–	3.0	6.8	–	9.0	2.7	8.2	0.2	0.6
Number of teeth on the collar	10	7	–	12		–		10.3	–	1.7	–
Claw 1 heights											
Branch	10	7.3	–	9.3	23.4	–	27.8	8.7	26.2	0.6	1.4
Spur	9	1.3	–	1.7	3.7	–	5.4	1.6	4.8	0.1	0.5
Spur/branch length ratio	9	16%	–	22%		–		18%	–	2%	–
Claw 2 heights											
Branch	10	7.2	–	8.6	22.0	–	26.5	8.0	24.1	0.5	1.4
Spur	7	1.3	–	1.9	4.2	–	5.9	1.5	4.6	0.2	0.6
Spur/branch length ratio	7	17%	–	22%		–		19%	–	2%	–
Claw 3 heights											
Branch	10	7.7	–	8.7	22.0	–	26.9	8.2	24.8	0.4	1.5
Spur	7	1.4	–	1.7	4.0	–	5.4	1.5	4.7	0.1	0.5
Spur/branch length ratio	7	17%	–	21%		–		19%	–	2%	–
Claw 4 heights											
Branch	10	8.5	–	10.9	26.3	–	33.2	9.9	29.7	0.7	1.9
Spur	6	1.7	–	2.4	4.8	–	7.4	2.0	6.0	0.3	0.9
Spur/branch length ratio	6	17%	–	24%		–		21%	–	3%	–

Table 8. Measurements [in µm] of selected morphological structures of larvae of *Echiniscus tropicalis* (population ID.939) mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD	
		µm			*sp*			µm	*sp*	µm	*sp*
Body length	3	110	–	124	559	–	574	119	567	8	7
Scapular plate length	3	19.4	–	22.0		–		21.0	–	1.4	–
Head appendages lengths											
Cirrus *internus*	3	4.2	–	4.6	20.9	–	21.6	4.5	21.3	0.2	0.4
Cephalic papilla	3	3.7	–	4.1	18.5	–	19.1	3.9	18.7	0.2	0.3
Cirrus *externus*	2	6.0	–	6.4	29.6	–	30.9	6.2	30.3	0.3	0.9
Clava	3	2.6	–	3.1	13.4	–	14.4	2.9	13.9	0.3	0.5
Cirrus *A*	3	13.3	–	15.5	60.9	–	71.8	14.1	67.1	1.2	5.6
Cirrus *A*/Body length ratio	3	11%	–	13%		–		12%	–	1%	–
Body appendages lengths											
Spine C^{d}	3	0.9	–	3.2	4.1	–	15.5	2.4	11.5	1.3	6.4
Spine D^{d}	3	3.2	–	6.0	16.5	–	27.8	4.8	22.8	1.5	5.8
Spine *E*	3	3.9	–	5.5	20.1	–	25.0	4.7	22.4	0.8	2.5
Spine on leg I length	3	1.2	–	1.6	6.2	–	7.4	1.5	7.0	0.2	0.7
Papilla on leg IV length	3	1.7	–	2.0	8.8	–	9.1	1.9	8.9	0.2	0.2
Number of teeth on the collar	3	6	–	7		–		6.7	–	0.6	–
Claw 1 heights											
Branch	3	5.3	–	5.8	26.4	–	27.3	5.6	26.7	0.3	0.5
Spur	3	1.1	–	1.4	5.7	–	6.5	1.3	6.2	0.2	0.4
Spur/branch length ratio	3	21%	–	25%		–		23%	–	2%	–
Claw 2 heights											
Branch	3	5.1	–	5.5	24.5	–	26.3	5.3	25.4	0.2	0.9
Spur	3	1.0	–	1.1	4.6	–	5.7	1.1	5.1	0.1	0.5
Spur/branch length ratio	3	18%	–	22%		–		20%	–	2%	–
Claw 3 heights											
Branch	3	5.1	–	5.7	24.5	–	26.4	5.4	25.7	0.3	1.0
Spur	3	1.2	–	1.3	5.5	–	6.2	1.2	5.9	0.1	0.4
Spur/branch length ratio	3	22%	–	24%		–		23%	–	1%	–
Claw 4 heights											
Branch	3	6.0	–	6.1	27.7	–	31.4	6.1	29.0	0.1	2.1
Spur	2	1.2	–	1.6	5.5	–	7.4	1.4	6.4	0.3	1.4
Spur/branch length ratio	2	20%	–	27%		–		23%	–	5%	–

sp. nov. vs *B* 4.3–12.7 µm, *C* 6.5–14.7 µm, *D* 5.7–13.8 µm in *E. tropicalis*);
- the presence of males (present in *E. insularis* sp. nov. vs absent in *E. tropicalis*);
- additionally, the spine *E* is frequently serrated in *E. tropicalis* (smooth in *E. insularis* sp. nov.).

Echiniscus perarmatus Murray, 1907

Material. Single adult female and a juvenile used for DNA sequencing (juvenile retrieved as a hologenophore on slide MU.001.23), larva on slide MU.001.01.

Remarks. This pantropical species (McInnes 1994) will likely appear to be one of the most common members of Echiniscidae in tropical climates, providing that the conspecificity of populations originating from different continents is demonstrated (data in preparation).

Genus: *Pseudechiniscus* Thulin, 1911
Subgenus: *Meridioniscus* Gąsiorek et al., 2021

Pseudechiniscus mascarenensis sp. nov. Kiosya, Vončina & Gąsiorek

http://zoobank.org/E5E6328D-D431-4BEA-88CB-0C24F6172B65

Figures 12–17, Tables 9–12

Pseudechiniscus sp. 5 in Gąsiorek et al. (2021)

***Locus typicus* and type material.** ca. 20°22'S, 57°29'E, 580 m asl; Sophie Nature Walk, vicinity of Mare aux Vacoas (Plaines Wilhems, Mauritius, Mascarene Archipelago, Western Indian Ocean); mosses from tree trunks. Holotype (mature female on slide MU.001.04), allotype (mature male on slide MU.001.05), sixty paratypic females, seven paratypic males, ten juveniles, and six larvae (slides MU.001.01–21). Single hologenophore on slide MU.001.22. All deposited in the Department of Invertebrate Evolution.

Etymology. The name indicates the Mascarenes, *terra typica* of the new species. Adjective in the nominative singular.

Description. Mature females (i.e. from the third instar onwards; measurements in Table 9). Body yellow to orange, with minute, round black eyes absent after mounting (Fig. 12A). Elongated (dactyloid) cephalic papillae (secondary clavae) and elongated (primary) clavae (Figs 12A, 13, 15, 16); peribuccal cirri with poorly developed cirrophores. Cirrus *A* short, with cirrophore.

Dorsal plates are both poorly sclerotised and demarcated from each other, with the *Pseudechiniscus*-type sculpturing, i.e. endocuticular pillars protruding through the epicuticle and visible as dark dots in PCM (Fig. 12A). *Striae* present, but not visible in SEM (Figs 15A, 16). Epicuticular ornamentation absent. The cephalic plate pentapartite, with the anterior bi-halved portion and three posterior portions, roughly equal in size. The cervical (neck) plate absent. The scapular plate with sutures, separating wide anterior portion and four rectangular posterior portions. Three median plates: m1 and m3 unipartite, the latter indistinctly merged with the anterior margin of the pseudosegmental plate IV' (Fig. 12A), clearly delimited in SEM (Fig. 15A); m2 bipartite and

Table 9. Measurements [in µm] of selected morphological structures of mature females of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD		Holotype	
		µm			*sp*			µm	*sp*	µm	*sp*	µm	*sp*
Body length	10	151	–	177	*621*	–	*843*	163	*712*	9	*64*	167	*732*
Scapular plate length	10	21.0	–	24.8		–		23.0	–	1.4	–	22.8	–
Head appendages lengths													
Cirrus *internus*	10	5.4	–	7.6	*23.3*	–	*32.9*	6.7	*29.2*	0.6	*2.9*	6.8	*29.8*
Cephalic papilla	10	4.3	–	5.7	*17.3*	–	*25.2*	5.0	*21.8*	0.5	*2.3*	4.4	*19.3*
Cirrus *externus*	10	7.7	–	13.0	*33.2*	–	*56.5*	10.8	*47.3*	1.6	*7.7*	11.5	*50.4*
Clava	10	3.4	–	5.2	*13.9*	–	*21.2*	4.2	*18.2*	0.5	*2.3*	4.0	*17.5*
Cirrus *A*	9	13.8	–	23.4	*58.1*	–	*95.5*	18.9	*82.9*	3.0	*14.6*	20.5	*89.9*
Cirrus *A*/Body length ratio	9	9%	–	13%		–		12%	–	2%	–	12%	–
Papilla on leg IV length	10	1.5	–	2.4	*7.0*	–	*11.0*	2.0	*8.9*	0.3	*1.2*	2.1	*9.2*
Claw 1 heights													
Branch	10	6.9	–	8.6	*29.7*	–	*39.5*	7.8	*34.1*	0.6	*3.0*	8.0	*35.1*
Spur	9	1.3	–	2.1	*5.7*	–	*8.6*	1.6	*7.2*	0.3	*1.0*	1.3	*5.7*
Spur/branch length ratio	9	16%	–	25%		–		21%	–	3%	–	16%	–
Claw 2 heights													
Branch	10	6.8	–	8.7	*29.3*	–	*38.6*	7.8	*33.9*	0.6	*2.9*	7.8	*34.2*
Spur	9	1.2	–	1.8	*5.2*	–	*7.6*	1.5	*6.4*	0.2	*0.8*	1.4	*6.1*
Spur/branch length ratio	9	17%	–	25%		–		19%	–	3%	–	18%	–
Claw 3 heights													
Branch	10	6.5	–	8.4	*28.9*	–	*40.0*	7.6	*33.3*	0.7	*3.2*	7.6	*33.3*
Spur	8	1.2	–	1.7	*5.6*	–	*7.2*	1.5	*6.4*	0.2	*0.5*	1.4	*6.1*
Spur/branch length ratio	8	18%	–	24%		–		20%	–	2%	–	18%	–
Claw 4 heights													
Branch	10	7.5	–	9.1	*30.2*	–	*42.4*	8.3	*36.2*	0.6	*3.4*	8.0	*35.1*
Spur	6	1.3	–	2.2	*6.0*	–	*9.0*	1.8	*7.6*	0.4	*1.3*	1.8	*7.9*
Spur/branch length ratio	6	17%	–	25%		–		21%	–	3%	–	23%	–

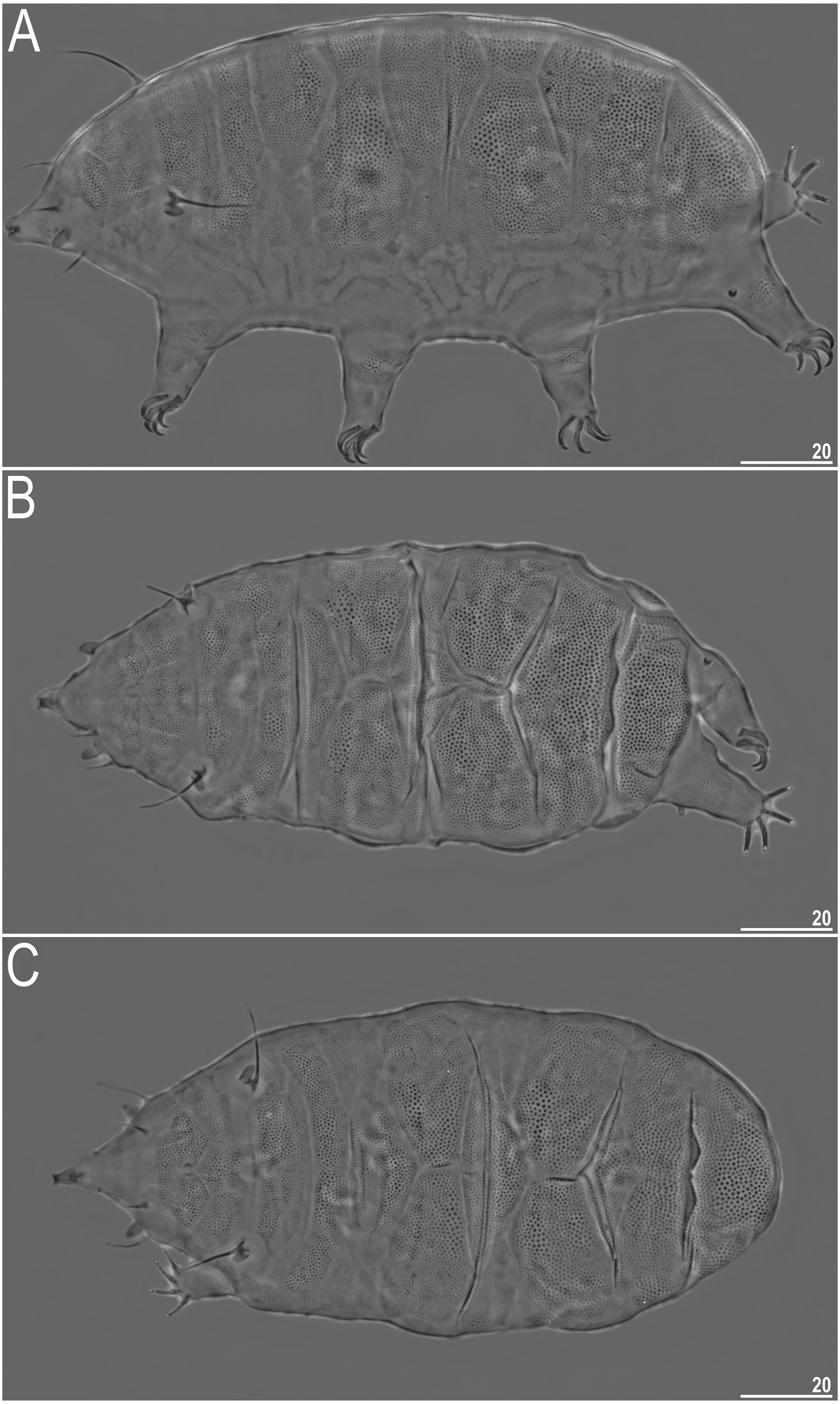

Figure 12. Habitus of *Pseudechiniscus mascarenensis* sp. nov. (PCM): **A** female, dorsolateral view, **B, C** males, dorsal view. Scale bars in μm.

Table 10. Measurements [in µm] of selected morphological structures of mature males of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD		Allotype	
		µm			*sp*			µm	*sp*	µm	*sp*	µm	*sp*
Body length	6	118	–	146	*605*	–	*670*	137	*650*	10	*24*	146	*652*
Scapular plate length	6	17.6	–	22.4		–		21.1	–	1.8	–	22.4	–
Head appendages lengths													
Cirrus *internus*	6	5.1	–	7.9	*23.9*	–	*36.9*	6.4	*30.7*	1.0	*5.1*	7.9	*35.3*
Cephalic papilla	6	3.5	–	4.9	*19.9*	–	*23.0*	4.5	*21.2*	0.5	*1.1*	4.6	*20.5*
Cirrus *externus*	5	8.0	–	10.0	*37.2*	–	*52.3*	9.3	*44.9*	0.8	*5.5*	?	*?*
Clava	6	3.5	–	4.6	*18.3*	–	*21.6*	4.1	*19.4*	0.3	*1.2*	4.1	*18.3*
Cirrus *A*	5	13.8	–	18.8	*73.2*	–	*85.9*	16.8	*79.9*	2.0	*5.2*	16.4	*73.2*
Cirrus *A*/Body length ratio	5	11%	–	14%		–		12%	–	1%	–	11%	–
Papilla on leg IV length	6	1.7	–	2.4	*7.6*	–	*11.2*	2.0	*9.5*	0.2	*1.2*	1.7	*7.6*
Claw 1 heights													
Branch	6	6.7	–	8.3	*30.9*	–	*38.1*	7.4	*35.1*	0.6	*2.8*	8.3	*37.1*
Spur	3	1.0	–	1.5	*4.7*	–	*7.0*	1.3	*6.0*	0.3	*1.2*	?	*?*
Spur/branch length ratio	3	14%	–	20%		–		18%	–	3%	–	?	–
Claw 2 heights													
Branch	6	6.2	–	7.5	*29.1*	–	*35.2*	6.9	*33.0*	0.5	*2.3*	7.2	*32.1*
Spur	5	1.1	–	1.5	*5.2*	–	*7.4*	1.3	*6.3*	0.2	*1.0*	1.5	*6.7*
Spur/branch length ratio	5	15%	–	21%		–		19%	–	3%	–	21%	–
Claw 3 heights													
Branch	6	6.3	–	7.2	*29.6*	–	*35.8*	6.8	*32.6*	0.4	*2.1*	7.1	*31.7*
Spur	3	1.2	–	1.5	*5.4*	–	*6.7*	1.3	*6.1*	0.2	*0.7*	1.5	*6.7*
Spur/branch length ratio	3	18%	–	21%		–		19%	–	2%	–	21%	–
Claw 4 heights													
Branch	6	6.6	–	8.1	*31.3*	–	*37.7*	7.5	*35.9*	0.6	*2.8*	7.0	*31.3*
Spur	2	1.5	–	1.5	*6.7*	–	*7.0*	1.5	*6.8*	0.0	*0.2*	1.5	*6.7*
Spur/branch length ratio	2	19%	–	21%		–		20%	–	2%	–	21%	–

large. Four pairs of lateral intersegmental platelets flanking the boarders of m1–2 (Figs 12A, 16). Two pairs of large segmental plates. The pseudosegmental plate IV' undivided by a median longitudinal suture; the posterior margin of the plate sinusoid and smooth. The caudal (terminal) plate with short, very poorly marked incisions (Figs 15A, 16).

Ventral cuticle with a pronounced species-specific pattern reaching the lateroventral sides of the body (Figs 12A, 13, 14, 15B), being a typical reticulum composed of belts of pillars. The pattern is relatively stable and well developed in the majority of individuals. The subcephalic zone with a wide belt of pillars. No epicuticular thickenings. Sexpartite gonopore located anterior to legs IV and a trilobed anus between legs IV.

Pedal plates and dentate collar IV absent; instead large patches of pillars are present centrally on each leg (Fig. 12A). Pulvini faint. Papilla on leg I absent (Figs 12A, 13, 16) and papilla on leg IV present (Figs 12A, 16, 17D). Claws I–IV of similar heights. External claws on all legs smooth. Internal claws with spurs positioned at ca. 1/5 of the claw height and directed downwards (Fig. 17).

Mature males (i.e. from the second or third instar onwards; measurements in Table 10). Clearly smaller than females (compare Tables 9, 10). The posterior margin of the pseudosegmental plate IV' bears two weakly developed lobes joined at their bases (Fig. 12B, C). Gonopore circular.

Juveniles (i.e. from the second instar onwards; measurements in Table 11). Morphometric gap exists between adult females and juveniles. Qualitatively similar to adults. Gonopore absent.

Larvae (i.e. the first instar; measurements in Table 12). Morphometric gap exists between juveniles and larvae. Gonopore and anus absent.

Eggs. One egg per exuviae was found in few examined exuviae.

DNA sequences and phylogenetic position. Single haplotypes in 18S rRNA (MW031972), 28S rRNA (MW032061), and ITS-1 (MW032151) were found. *Pseudechiniscus mascarenensis* sp. nov. has no close relatives according to the phylogeny presented in Gąsiorek et al. (2020) (see fig. 2 therein), constituting a separate evolutionary lineage within the subgenus *Meridioniscus*.

Phenotypic differential diagnosis. The species must be compared to other members of *Meridioniscus* with no projections on the pseudosegmental plate IV' or with rudimentarily developed projections. *Pseudechiniscus mascarenensis* sp. nov. is differentiated from:

- *P. angelusalas* Roszkowska et al., 2020, described from Madagascar, by the body length (151–177 µm in females of *P. mascarenensis* sp. nov. vs 113–143 µm in females of *P. angelusalas*), the cirrus *A*/body length ratio (9–13% in females of *P. mascarenensis* sp. nov. vs 19–22% in females of *P. angelusalas*), and by the division of the pseudosegmental plate IV' (undivided in *P. mascarenensis* sp. nov. vs with median longitudinal suture in *P. angelusalas*);
- *P. dastychi* Roszkowska et al., 2020, described from the Argentine Islands (maritime Antarctic), by the presence of males (present in *P. mascarenensis* sp. nov. vs absent in *P. dastychi*), and by the division of the pseudosegmental plate IV' (undivided in

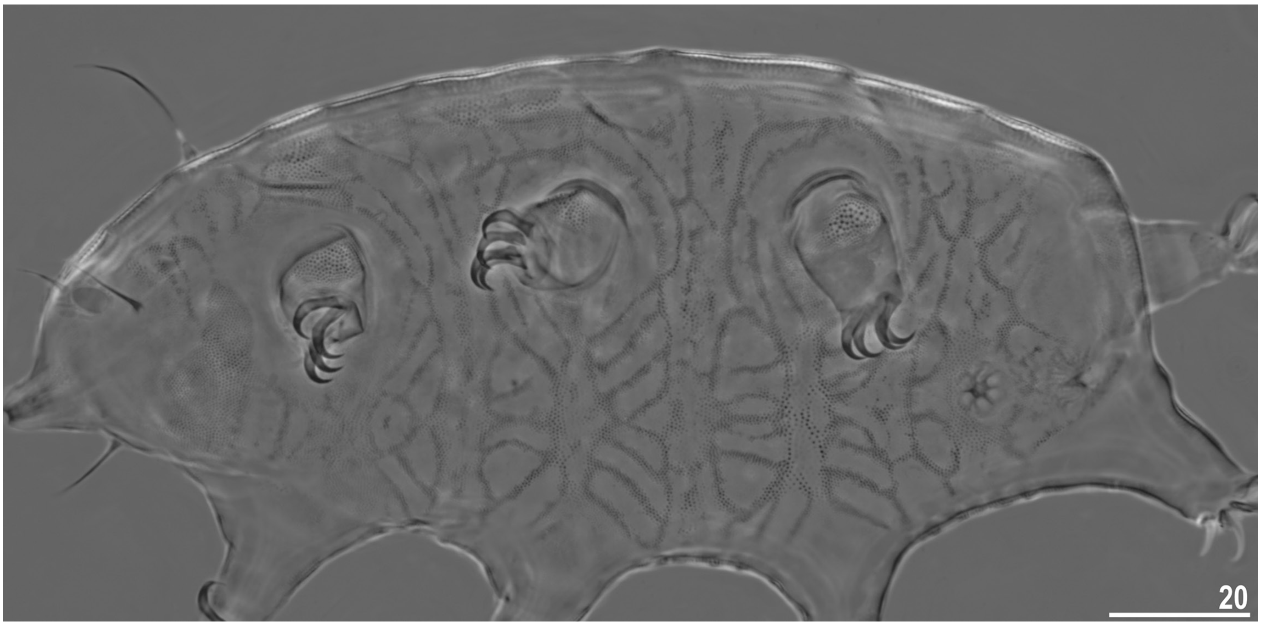

Figure 13. Ventral sculpturing pattern of female of *Pseudechiniscus mascarenensis* sp. nov. (PCM). Scale bar in µm.

Table 11. Measurements [in µm] of selected morphological structures of juveniles of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD	
		µm			*sp*			µm	*sp*	µm	*sp*
Body length	5	115	–	129	*635*	–	*726*	124	*672*	5	*33*
Scapular plate length	5	17.5	–	19.3		–		18.4	–	0.7	–
Head appendages lengths											
Cirrus *internus*	5	5.2	–	6.9	*27.8*	–	*38.1*	5.9	*32.3*	0.7	*4.4*
Cephalic papilla	5	3.4	–	3.9	*17.6*	–	*21.2*	3.5	*19.3*	0.2	*1.4*
Cirrus *externus*	5	6.9	–	7.8	*38.1*	–	*42.3*	7.5	*40.7*	0.4	*1.6*
Clava	5	2.8	–	3.9	*15.0*	–	*21.2*	3.3	*18.0*	0.5	*2.7*
Cirrus *A*	5	12.7	–	16.4	*65.8*	–	*89.1*	14.3	*77.7*	1.9	*10.0*
Cirrus *A*/Body length ratio	5	10%	–	13%		–		12%	–	2%	–
Papilla on leg IV length	4	1.5	–	2.0	*8.0*	–	*10.9*	1.8	*9.5*	0.3	*1.4*
Claw 1 heights											
Branch	5	6.1	–	6.4	*33.2*	–	*34.9*	6.3	*34.0*	0.1	*0.8*
Spur	2	1.2	–	1.3	*6.2*	–	*7.4*	1.3	*6.8*	0.1	*0.9*
Spur/branch length ratio	2	19%	–	21%		–		20%	–	2%	–
Claw 2 heights											
Branch	5	5.2	–	5.8	*28.0*	–	*31.5*	5.6	*30.3*	0.3	*1.5*
Spur	3	1.3	–	1.4	*6.7*	–	*7.6*	1.3	*7.1*	0.1	*0.5*
Spur/branch length ratio	3	22%	–	24%		–		24%	–	1%	–
Claw 3 heights											
Branch	5	5.6	–	6.4	*29.5*	–	*35.4*	6.0	*32.5*	0.3	*2.6*
Spur	3	1.1	–	1.3	*5.7*	–	*7.1*	1.2	*6.5*	0.1	*0.7*
Spur/branch length ratio	3	19%	–	21%		–		20%	–	1%	–
Claw 4 heights											
Branch	5	6.4	–	6.9	*33.2*	–	*37.7*	6.6	*36.0*	0.2	*1.9*
Spur	2	1.1	–	1.2	*6.0*	–	*6.6*	1.2	*6.3*	0.1	*0.5*
Spur/branch length ratio	2	16%	–	18%		–		17%	–	2%	–

P. mascarenensis sp. nov. vs with median longitudinal suture in *P. dastychi*);

- *P. indistinctus* Roszkowska et al., 2020, described from Norway, by the division of the pseudosegmental plate IV' (undivided in *P. mascarenensis* sp. nov. vs with median longitudinal suture in *P. indistinctus*), and by the presence of males (present in *P. mascarenensis* sp. nov. vs absent in *P. indistinctus*);
- *P. santomensis* Fontoura et al., 2010, considered endemic to the island São Tomé (Gulf of Guinea), by the presence of males (present in *P. mascarenensis* sp. nov. vs absent in *P. santomensis*), and the morphology of the posterior margin of pseudosegmental plate IV' in females (smooth in *P. mascarenensis* sp. nov. vs with two projections in *P. santomensis*, as in males of *P. mascarenensis* sp. nov.);

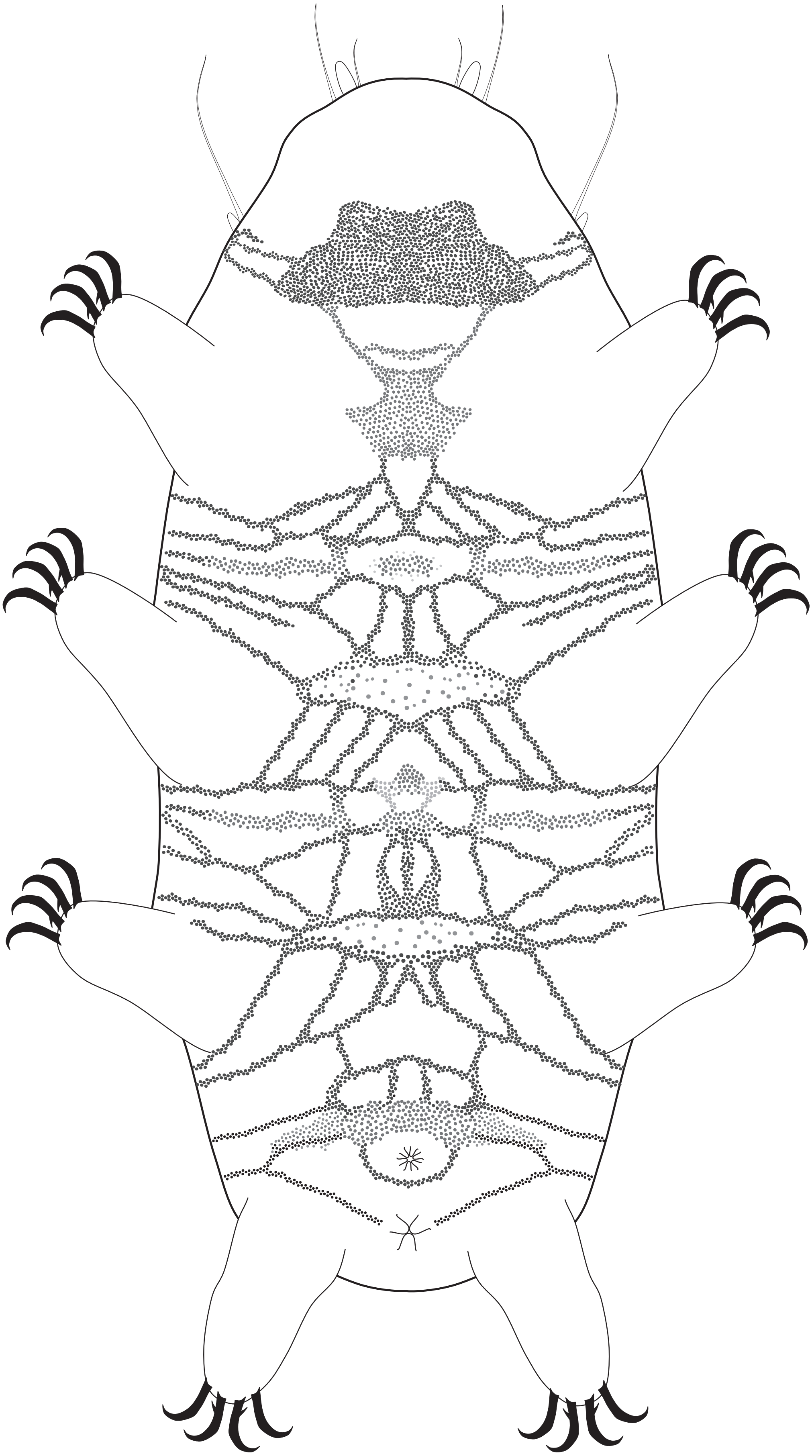

Figure 14. Schematic ventral sculpturing pattern of female of *Pseudechiniscus mascarenensis* sp. nov.

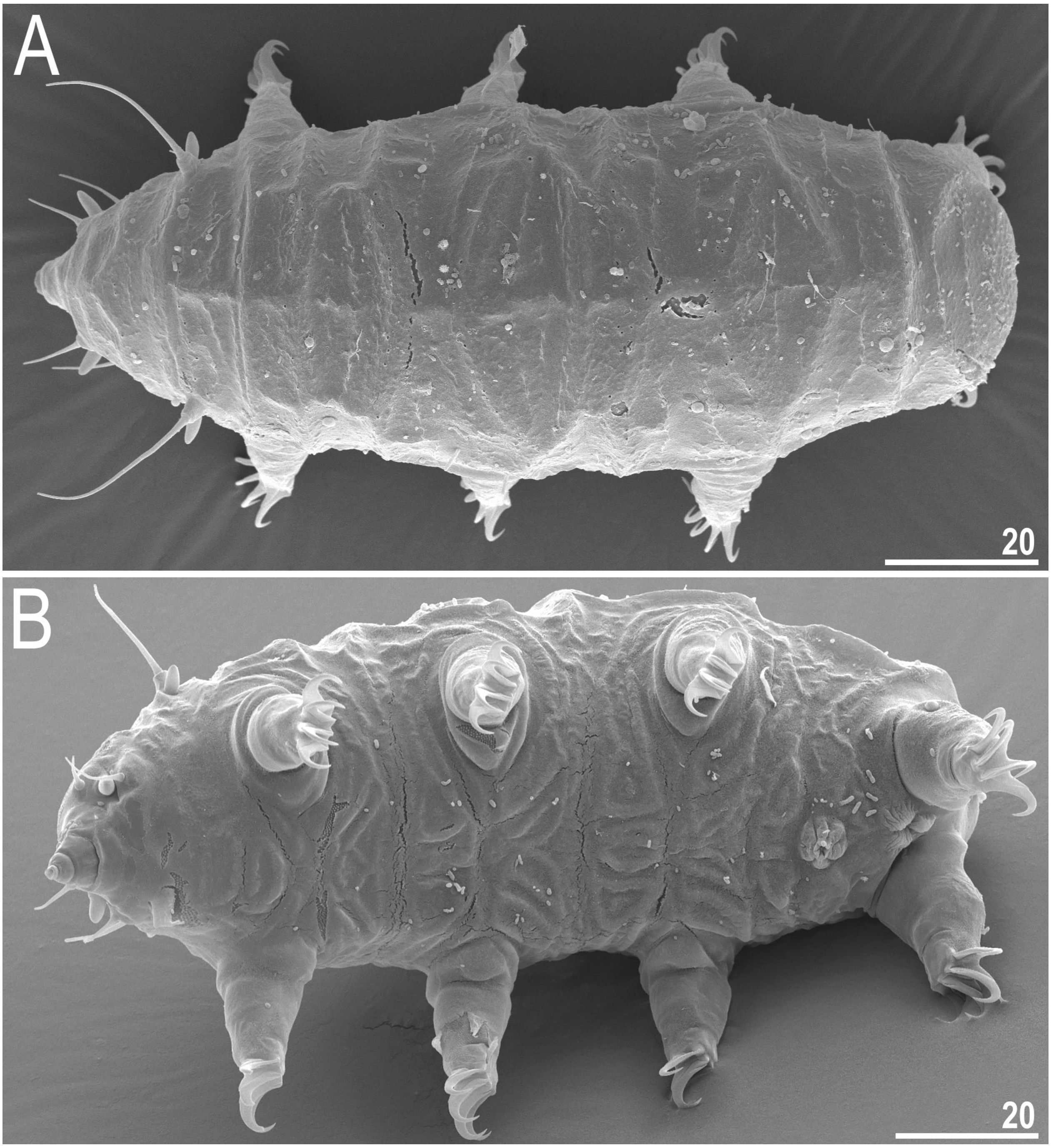

Figure 15. Habitus of females of *Pseudechiniscus mascarenensis* sp. nov. (SEM): **A** dorsal view, **B** ventral view. Scale bars in μm.

overall, these two species are most similar within the genus.

Moreover, only the ventral sculpturing pattern of *P. santomensis* resembles that of *P. mascarenensis* sp. nov.; the remaining species have a very different ventral arrangement of pillars. *Pseudechiniscus juanitae* de Barros, 1939 should be treated as unidentifiable due to the lack of knowledge on its morphology (Grobys et al. 2020), although one attempt was made to characterise this species based on individuals from Central America (Pilato and Lisi 2006; Tumanov 2020), whereas its *locus typicus* lies in Brazil. Consequently, it is not included within the differential diagnosis.

Discussion

The fauna of Mauritius has previously been illustrative for an isolated oceanic island, consisting of a small number of mostly endemic species (Cheke and Hume 2008; Kehlmaier et al. 2019). However, many native species have been extirpated and replaced by allochthonous, often invasive taxa, a process which has been documented for numerous islands (Drake et al. 2002). Although the interactions between tardigrades inhabiting a given microhabitat are poorly understood (Meyer et al. 2020), in some cases it is easy to pinpoint with high level of certainty that a species is not native to a region. For example, *Echiniscus testudo* (Doyère, 1840) was report-

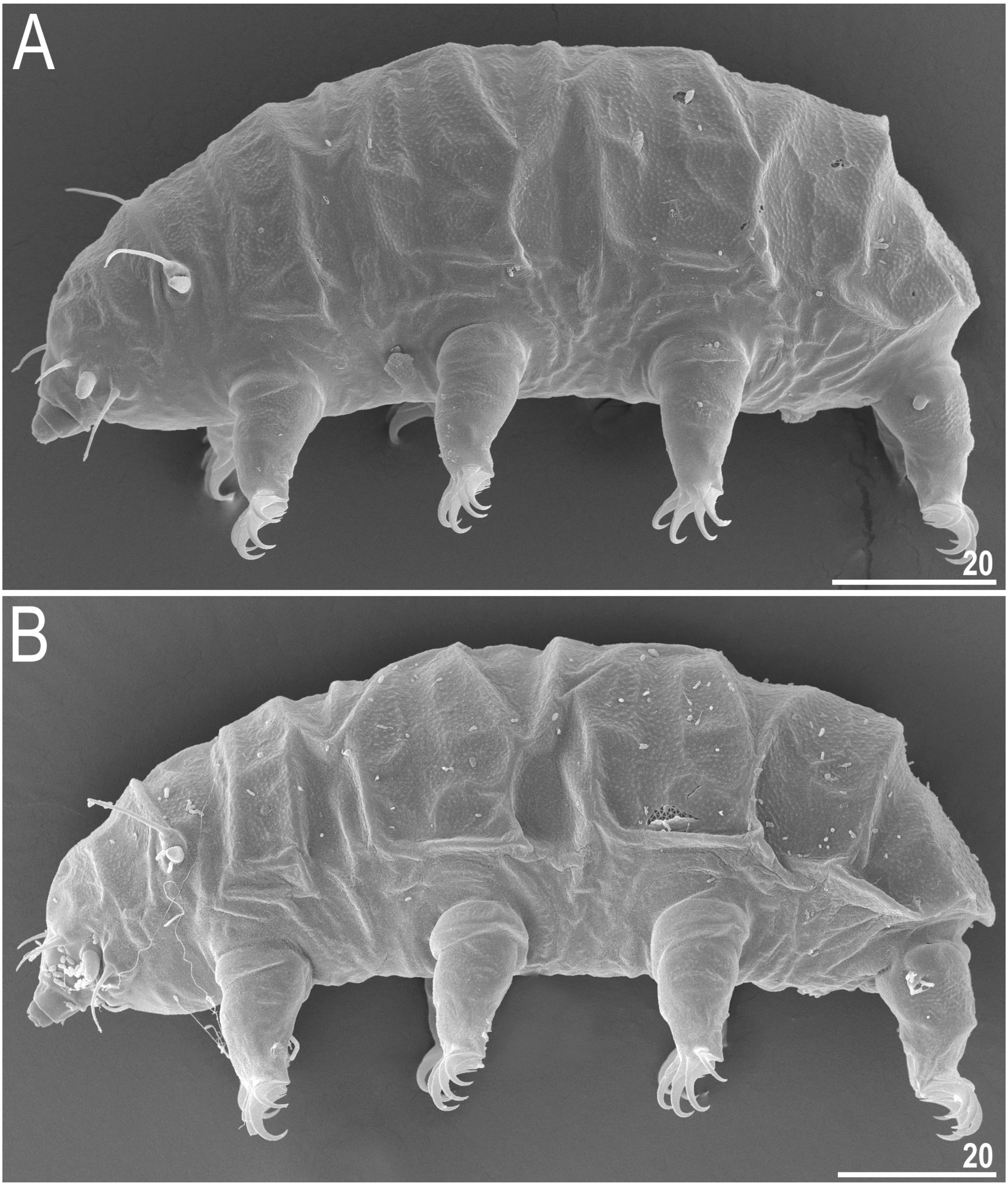

Figure 16. Habitus of females of *Pseudechiniscus mascarenensis* sp. nov. (SEM) in lateral view. Scale bars in μm.

ed from the Seychelles (Pilato et al. 2002), and it is highly probable that it was brought there by the Europeans during the colonialism era. The impact of such successfully colonising tardigrade species on local communities is unknown.

Our contribution provides first faunistic data on limno-terrestrial tardigrades for Mauritius, and reveals one species (*E. perarmatus*) probably widely distributed in the tropics (McInnes 1994). This concurs with pantropical records of another echiniscid, *E. lineatus* (Gąsiorek et al. 2019), and the wide geographic range of *E. tropicalis* (Fig. 18). The remaining two species are potentially endemic to the island, and the morphological similarity of *E. insularis* sp. nov. and *E. tropicalis* (Binda and Pilato 1995) would suggest that autochthonous tardigrades inhabiting Mauritius and Seychelles likely share a common origin. However, *E. tropicalis* is not closely related to *E. insularis* sp. nov. since its immediate kin is *E. siticulosus* (Fig. 8), an Australian endemic. Niedbała (2017) showed that oribatid mites of the Madagascan region are mostly endemic, but, at the same time, its fauna is more similar to the Afrotropical than to

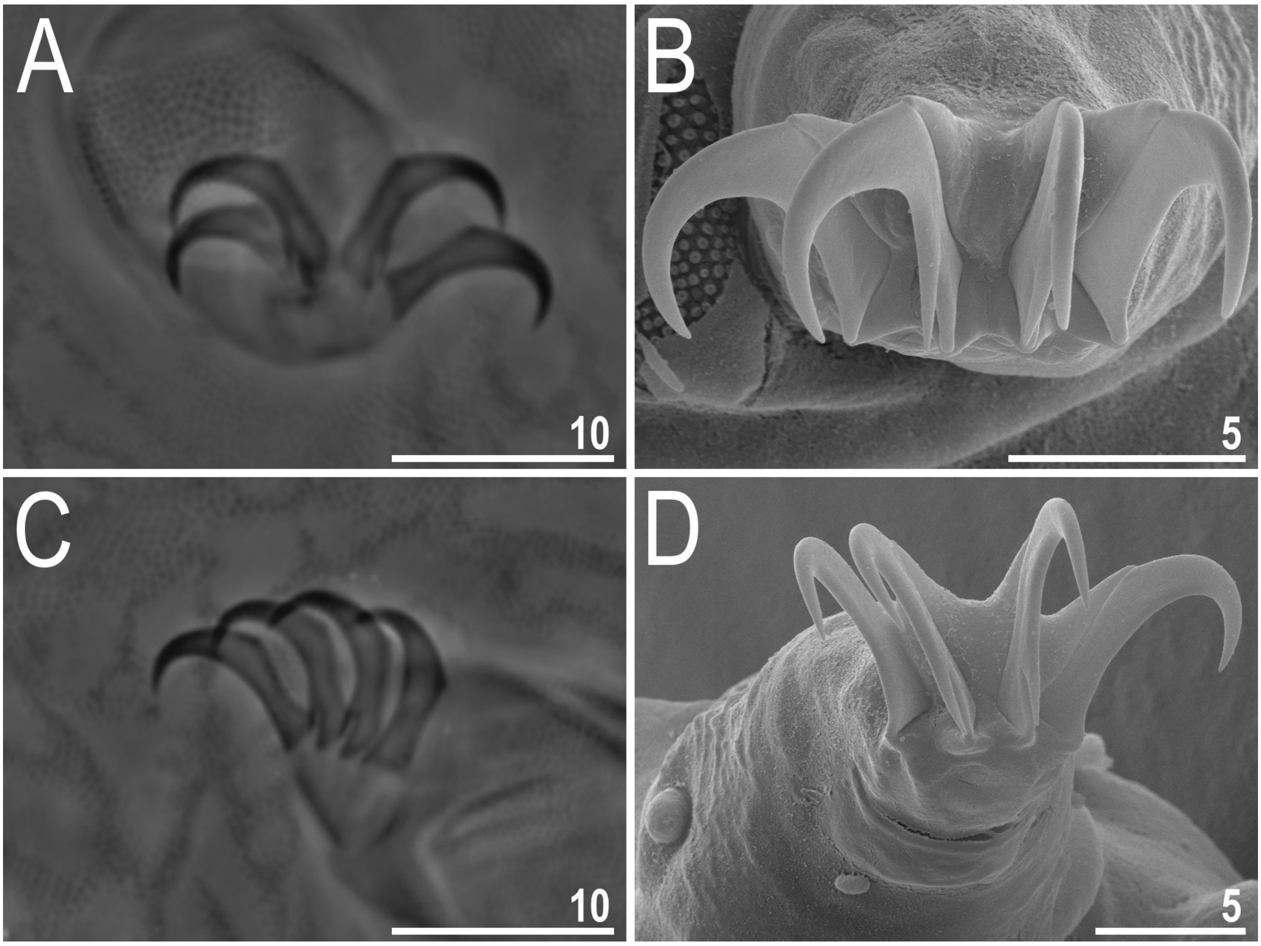

Figure 17. Claws of *Pseudechiniscus mascarenensis* sp. nov.: **A** claws I (PCM), **B** claws II (SEM), **C** claws III (PCM), **D** claws IV with papilla (SEM). Scale bars in µm.

Table 12. Measurements [in µm] of selected morphological structures of larvae of *Pseudechiniscus mascarenensis* sp. nov. mounted in Hoyer's medium (N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation).

Character	N	Range						Mean		SD	
		µm			*sp*			µm	*sp*	µm	*sp*
Body length	5	89	–	105	*603*	–	*699*	99	*643*	6	*43*
Scapular plate length	5	14.3	–	17.4		–		15.5	–	1.4	–
Head appendages lengths											
Cirrus *internus*	4	3.7	–	5.2	*25.9*	–	*30.6*	4.5	*28.7*	0.6	*2.1*
Cephalic papilla	5	2.8	–	3.6	*17.2*	–	*25.2*	3.2	*21.1*	0.4	*3.6*
Cirrus *externus*	5	5.2	–	6.6	*31.9*	–	*45.5*	5.7	*37.3*	0.6	*6.0*
Clava	5	2.5	–	3.4	*14.4*	–	*20.5*	2.7	*17.6*	0.4	*2.2*
Cirrus *A*	4	10.3	–	14.2	*59.8*	–	*99.3*	11.7	*75.8*	1.8	*18.6*
Cirrus *A*/Body length ratio	4	10%	–	14%		–		12%	–	2%	–
Papilla on leg IV length	4	1.1	–	1.5	*6.6*	–	*9.1*	1.3	*8.3*	0.2	*1.1*
Claw 1 heights											
Branch	5	5.0	–	5.9	*30.7*	–	*38.1*	5.4	*35.0*	0.4	*3.0*
Spur	4	1.0	–	1.6	*6.0*	–	*11.2*	1.2	*8.2*	0.3	*2.2*
Spur/branch length ratio	4	20%	–	30%		–		23%	–	4%	–
Claw 2 heights											
Branch	5	4.6	–	5.1	*28.7*	–	*35.0*	5.0	*32.2*	0.2	*2.7*
Spur	5	0.9	–	1.5	*6.1*	–	*10.5*	1.1	*7.4*	0.2	*1.8*
Spur/branch length ratio	5	18%	–	30%		–		23%	–	5%	–
Claw 3 heights											
Branch	5	4.7	–	5.3	*29.5*	–	*36.4*	5.0	*32.1*	0.3	*2.6*
Spur	2	0.9	–	1.0	*6.2*	–	*6.8*	1.0	*6.5*	0.1	*0.4*
Spur/branch length ratio	2	19%	–	21%		–		20%	–	2%	–
Claw 4 heights											
Branch	5	5.4	–	6.2	*32.5*	–	*43.4*	5.7	*37.1*	0.4	*4.0*
Spur	1	1.3	–	1.3	*8.8*	–	*8.8*	1.3	*8.8*	?	*?*
Spur/branch length ratio	1	24%	–	24%		–		24%	–	?	–

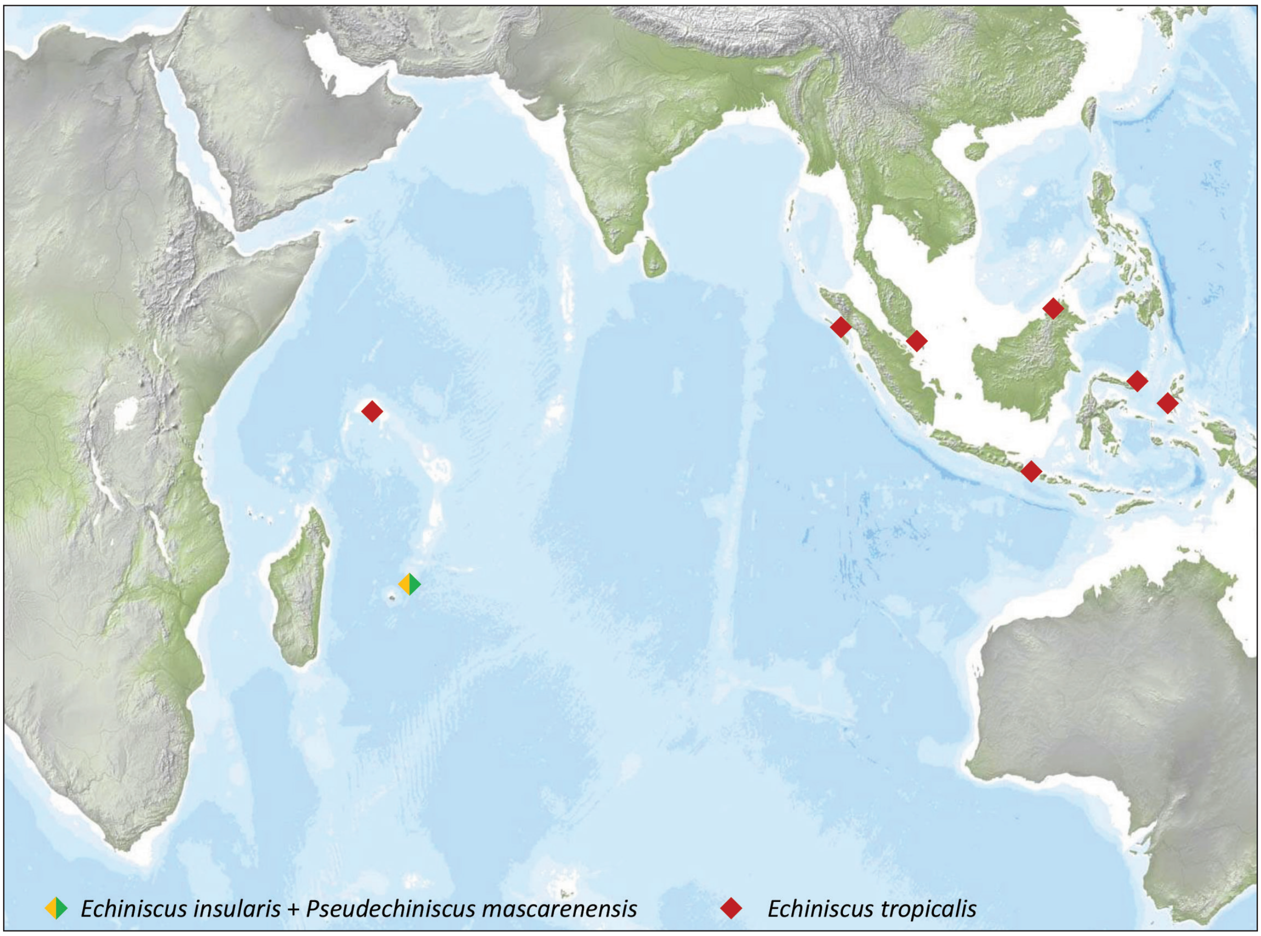

Figure 18. Distribution of species discussed in the present study. Map downloaded from www.freeworldmaps.net.

the Oriental realm. Given that the wind dispersal is an important movement mechanism for both oribatids and tardigrades (Lehmitz et al. 2011; Nelson et al. 2018; Gąsiorek et al. 2019), it is probable that the closest relatives of endemic Mauritian echiniscids should also be sought in Africa.

The supernumerary dorsal appendages of *E. insularis* sp. nov. are a morphological peculiarity, atypical for *Echiniscus*. Other species exhibiting appendages along the plate margins are very rare: *E. africanus*, *E. baloghi*, and *E. semifoveolatus* (Murray 1907; Iharos 1973; Ito 1993; Gąsiorek and Vončina 2019). These appendages, usually in the form of spicules, should be regarded as morphological convergence, appearing at the same time in the distantly related genus *Acanthechiniscus* (Vecchi et al. 2016). However, in the new Mauritian species, these spicules grow out of the surface of the dorsal plates, which is not present in any other echiniscids. Together with the small body size as for an *Echiniscus*, the presence of these spicules make *E. insularis* sp. nov. easily distinguishable and characteristic taxon. Contrarily, *P. mascarenensis* sp. nov. is a typical representative of *Pseudechiniscus*, more precisely of the subgenus *Meridioniscus*, which has been poorly represented in the molecular dataset under the term "*novaezeelandiae* group" (Cesari et al. 2020), until recently when *Pseudechiniscus* was divided into subgenera thanks to augmenting the phylogenetic inference for this genus based on a large molecular dataset (Gąsiorek et al. 2021). In summary, this limited sampling in the centre of Mauritius reflects the restricted knowledge about insular tardigrade faunas not only in the region of Indian Ocean, but throughout the world.

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References

Bartels PJ, Apodaca JJ, Mora C, Nelson DR (2016) A global biodiversity estimate of a poorly known taxon: phylum Tardigrada. Zoological Journal of the Linnean Society 178: 730–736. https://doi.org/10.1111/zoj.12441

Binda MG, Pilato G (1995) Remarks on tardigrades from Seychelles, with a description of two new species. Tropical Zoology 8: 1–6. https://doi.org/10.1080/03946975.1995.10539269

Biserov VI (1994) Some tardigrades from the Seychelles with description of three new species. Tropical Zoology 7: 181–189. https://doi.org/10.1080/03946975.1994.10539250

Blaxter M, Elsworth B, Daub J (2003) DNA taxonomy of a neglected animal phylum: an unexpected diversity of tardigrades. Proceedings of the Royal Society B 271(Supplement): 189–192. https://doi.org/10.1098/rsbl.2003.0130

Casquet J, Thebaud C, Gillespie RG (2012) Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. Molecular Ecology Resources 12: 136–141. https://doi.org/10.1111/j.1755-0998.2011.03073.x

Cesari M, Montanari M, Kristensen RM, Bertolani R, Guidetti R, Rebecchi L (2020) An integrated study of the biodiversity within the *Pseudechiniscus suillus–facettalis* group (Heterotardigrada: Echiniscidae). Zoological Journal of the Linnean Society 188: 717–732. https://doi.org/10.1093/zoolinnean/zlz045

Cheke A, Hume J (2008) Lost land of the dodo: The ecological history of Mauritius, Réunion, and Rodrigues. Yale University Press, New Haven.

Dabert J, Ehrnsberger R, Dabert M (2008) *Glaucalges tytonis* sp. nov. (Analgoidea: Xolalgidae) from the barn owl *Tyto alba* (Strigiformes: Tytonidae): compiling morphology with DNA barcode data for taxa descriptions in mites (Acari). Zootaxa 1719: 41–52. https://doi.org/10.11646/zootaxa.1719.1.2

da Cunha AX, do Nascimento Ribeiro F (1962) A fauna de tardígrados da Ilha da Madeira. Memórias e Estudos do Museu Zoológico da Universidade de Coimbra 279: 1–24.

Dastych H (1980) Niesporczaki (Tardigrada) Tatrzańskiego Parku Narodowego. Monografie Fauny Polski 9: 1–232.

Dastych H (1999) A new species of the genus *Mopsechniscus* Du Bois-Reymond Marcus, 1944 (Tardigrada) from the Venezuelan Andes. Acta biologica Benrodis 10: 91–101.

de Barros R (1939) *Pseudechiniscus juanitae*, nova espécie de tardigrado. Boletim Biológico, New Series 4: 367–368.

De Zio Grimaldi S, Lamarca A, Gallo D'Addabbo M, Pietanza R (1999) Florarctinae of Asdhu Island, Maldives, Indian Ocean (Tardigrada, Heterotardigrada). Italian Journal of Zoology 66: 383–391. https://doi.org/10.1080/11250009909356282

Degma P (2018) Field and laboratory methods. In: Schill R (Ed.) Water bears: The biology of tardigrades. Zoological Monographs, 2, chapter 14. Springer, Cham, 349–369. https://doi.org/10.1007/978-3-319-95702-9_14

Doyère M (1840) Mémoire sur les Tardigrades. Annales des Sciences Naturalles, Zoologia, Paris, Series 2, 14: 269–362.

Drake DR, Mulder CPH, Towns DR, Daugherty CH (2002) The biology of insularity: an introduction. Journal of Biogeography 29: 563–569. https://doi.org/10.1046/j.1365-2699.2002.00706.x

Faurby S, Jørgensen A, Kristensen RM, Funch P (2012) Distribution and speciation in marine intertidal tardigrades: testing the roles of climatic and geographical isolation. Journal of Biogeography 39: 1596–1607. https://doi.org/10.1111/j.1365-2699.2012.02720.x

Fontoura P, Pilato G, Lisi O (2010) First record of Tardigrada from São Tomé (Gulf of Guinea, Western Equatorial Africa) and description of *Pseudechiniscus santomensis* sp. nov. (Heterotardigrada: Echiniscidae). Zootaxa 2564: 31–42. https://doi.org/10.11646/zootaxa.2564.1.2

Gąsiorek P, Degma P (2018) Three Echiniscidae species (Tardigrada: Heterotardigrada) new to the Polish fauna, with the description of a new gonochoristic *Bryodelphax* Thulin, 1928. Zootaxa 4410: 77–96. https://doi.org/10.11646/zootaxa.4410.1.4

Gąsiorek P, Jackson KJ, Meyer H, Zając K, Nelson DR, Kristensen RM, Michalczyk Ł (2019) *Echiniscus virginicus* complex: the first case of pseudocryptic allopatry and pantropical distribution in tardigrades. Biological Journal of the Linnean Society 128: 789–805. https://doi.org/10.1093/biolinnean/blz147

Gąsiorek P, Kristensen RM (2018) Echiniscidae (Heterotardigrada) of Tanzania and Uganda. Tropical Zoology 31: 131–160. https://doi.org/10.1080/03946975.2018.1477350

Gąsiorek P, Stec D, Morek W, Michalczyk Ł (2017) An integrative redescription of *Echiniscus testudo* (Doyère, 1840), the nominal taxon for the class Heterotardigrada (Ecdysozoa: Panarthropoda: Tardigrada). Zoologischer Anzeiger 270: 107–122. https://doi.org/10.1016/j.jcz.2017.09.006

Gąsiorek P, Stec D, Zawierucha K, Kristensen RM, Michalczyk Ł (2018) Revision of *Testechiniscus* Kristensen, 1987 (Heterotardigrada: Echiniscidae) refutes the polar-temperate distribution of the genus. Zootaxa 4472: 261–297. https://doi.org/10.11646/zootaxa.4472.2.3

Gąsiorek P, Vončina K (2019) New Echiniscidae (Heterotardigrada) from Amber Mountain (Northern Madagascar). Evolutionary Systematics 3: 29–39. https://doi.org/10.3897/evolsyst.3.33580

Gąsiorek P, Vončina K, Zając K, Michalczyk Ł (2021) Phylogeography and morphological evolution of *Pseudechiniscus* (Heterotardigrada: Echiniscidae). Scientific Reports 11: 7606. https://doi.org/10.1038/s41598-021-84910-6

Goodman SM, Benstead JP (2005) Updated estimates of biotic diversity and endemism for Madagascar. Oryx 39: 73–77. https://doi.org/10.1017/S0030605305000128

Grimaldi De Zio S, D'Addabbo Gallo M, Morone de Lucia MR, Daddabbo L (1987) Marine Arthrotardigrada and Echiniscoidea (Tardigrada, Heterotardigrada) from the Indian Ocean. Bollettino di zoologia 54: 347–357. https://doi.org/10.1080/11250008709355608

Grobys D, Roszkowska M, Gawlak M, Kmita H, Kepel A, Kepel M, Parnikoza I, Bartylak T, Kaczmarek Ł (2020) High diversity in the *Pseudechiniscus suillus–facettalis* complex (Heterotardigrada: Echiniscidae) with remarks on the morphology of the genus *Pseudechiniscus*. Zoological Journal of the Linnean Society 188: 733–752. https://doi.org/10.1093/zoolinnean/zlz171

Guidetti R, Cesari M, Bertolani R, Altiero T, Rebecchi L (2019) High diversity in species, reproductive modes and distribution within the *Paramacrobiotus richtersi* complex (Eutardigrada, Macrobiotidae). Zoological Letters 5: 1. https://doi.org/10.1186/s40851-018-0113-z

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.1093/molbev/msx281

Iharos G (1973) Neuere Daten zur Kenntnis der Tardigraden-Fauna von Neuguinea. Opuscula Zoologica, Budapest 11: 65–73.

Ito M (1993) Taxonomic study on the class Heterotardigrada (Tardigrada) from the northern slope of Mt. Fuji, Central Japan. Edaphologia 50: 1–13.

Jørgensen A, Kristensen RM, Møbjerg N (2018) Phylogeny and integrative taxonomy of Tardigrada. In: Schill R (Ed.) Water bears: The biology of tardigrades. Zoological Monographs, 2, chapter 3. Springer, Cham, 95–114. https://doi.org/10.1007/978-3-319-95702-9_3

Kaczmarek Ł, Roszkowska M, Poprawa I, Janelt K, Kmita H, Gawlak M, Fiałkowska E, Mioduchowska M (2020) Integrative description of bisexual *Paramacrobiotus experimentalis* sp. nov. (Macrobiotidae) from Republic of Madagascar (Africa) with microbiome analysis. Molecular Phylogenetics and Evolution 145: 106730. https://doi.org/10.1016/j.ympev.2019.106730

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Kehlmaier C, Graciá E, Campbell PD, Hofmeyr MD, Schweiger S, Martínez-Silvestre A, Joyce W, Fritz U (2019) Ancient mitogenomics clarifies radiation of extinct Mascarene giant tortoises (*Cylindraspis* spp.). Scientific Reports 9: 17487. https://doi.org/10.1038/s41598-019-54019-y

Lehmitz R, Russell D, Hohberg K, Christian A, Xylander WER (2011) Wind dispersal of oribatid mites as a mode of migration. Pedobiologia 54: 201–207. https://doi.org/10.1016/j.pedobi.2011.01.002

Marcus E (1927) Zur Anatomie und Ökologie mariner Tardigraden. Zoologische Jahrbücher Abteilung für Systematik 53: 487–558.

McInnes SJ (1994) Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. Journal of Natural History 28: 257–352. https://doi.org/10.1080/00222939400770131

Meyer HA, Larsen HE, Akobi NO, Broussard G (2020) Predator and prey detection in two species of water bear (Tardigrada). Zoological Journal of the Linnean Society 188: 860–864. https://doi.org/10.1093/zoolinnean/zlz141

Michalczyk Ł, Kaczmarek Ł (2013) The Tardigrada Register: a comprehensive online data repository for tardigrade taxonomy. Journal of Limnology 72: 175–181. https://doi.org/10.4081/jlimnol.2013.s1.e22

Mironov SV, Dabert J, Dabert M (2012) A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the long-tailed tit *Aegithalos caudatus* (Passeriformes: Aegithalidae): morphological description with DNA barcode data. Zootaxa 3253: 54–61. https://doi.org/10.11646/zootaxa.3253.1.2

Morek W, Michalczyk Ł (2020) First extensive multilocus phylogeny of the genus *Milnesium* (Tardigrada) reveals no congruence between genetic markers and morphological traits. Zoological Journal of the Linnean Society 188: 681–693. https://doi.org/10.1093/zoolinnean/zlz040

Motala SM, Krell F-T, Mungroo Y, Donovan SE (2007) The terrestrial arthropods of Mauritius: a neglected conservation target. Biodiversity and Conservation 16: 2867–2881. https://doi.org/10.1007/s10531-006-9050-9

Murray J (1907) Some South African Tardigrada. Journal of the Royal Microscopical Society 27: 515–524. https://doi.org/10.1111/j.1365-2818.1907.tb01665.x

Nelson DR, Bartels PJ, Guil N (2018) Tardigrade ecology. In: Schill R (Ed.) Water bears: The biology of tardigrades. Zoological Monographs, 2, chapter 7. Springer, Cham, 163–210. https://doi.org/10.1007/978-3-319-95702-9_7

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Niedbała W (2017) Ptyctimous mites (Acari, Oribatida) of Madagascar and neighbouring islands. Acarologia 57: 3–205. https://doi.org/10.1051/acarologia/20164149

Pilato G, Binda MG, Lisi O (2002) Notes on tardigrades of the Seychelles with the description of two new species. Bollettino dell'Accademia Gioenia di Scienze Naturali, Catania 35: 503–517.

Pilato G, Binda MG, Lisi O (2004) Notes on some tardigrades of the Seychelles with the description of three new species. Italian Journal of Zoology 71: 171–178. https://doi.org/10.1080/11250000409356569

Pilato G, Binda MG, Lisi O (2006) Three new species of eutardigrades from the Seychelles. New Zealand Journal of Zoology 33: 39–48. https://doi.org/10.1080/03014223.2006.9518429

Pilato G, Lisi O (2006) Notes on some tardigrades from southern Mexico with description of three new species. Zootaxa 1236: 53–68. https://doi.org/10.11646/zootaxa.1236.1.4

Pilato G, Lisi O (2009a) Description of three new species of Tardigrada from the Seychelles. Zootaxa 2005: 24–34. https://doi.org/10.11646/zootaxa.2005.1.2

Pilato G, Lisi O (2009b) Tardigrades of the Seychelles Islands, with the description of three new species. Zootaxa 2124: 1–20. https://doi.org/10.11646/zootaxa.2124.1.1

Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, Sundberg P, Thollesson M (2008) Phylogenies without roots? A plea for the use of vouchers in molecular studies. Molecular Phylogenetics and Evolution 48: 369–371. https://doi.org/10.1016/j.ympev.2008.03.024

Richters F (1903) Nordische Tardigraden. Zoologischer Anzeiger 27: 168–172.

Richters F (1926) Tardigrada. In: Kükenthal W, Krumbach T (Eds) Handbuch der Zoologie. Vol. 3. Walter de Gruyter & Co., Berlin and Leipzig, 58–61 pp.

Roszkowska M, Grobys D, Bartylak T, Gawlak M, Kmita H, Kepel A, Kepel M, Parnikoza I, Kaczmarek Ł (2020) Integrative description of five *Pseudechiniscus* species (Heterotardigrada: Echiniscidae: the *suillus-facettalis* complex). Zootaxa 4763: 451–484. https://doi.org/10.11646/zootaxa.4763.4.1

Séméria Y (2003) Une espèce nouvelle de tardigrade pour l'Ile de La Reunion: *Cornechiniscus madagascariensis* Maucci. Bulletin mensuel de la Société linnéenne de Lyon 72: 233–234. https://doi.org/10.3406/linly.2003.13476

Stec D, Smolak R, Kaczmarek Ł, Michalczyk Ł (2015) An integrative description of *Macrobiotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya. Zootaxa 4052: 501–526. https://doi.org/10.11646/zootaxa.4052.5.1

Stec D, Zawierucha K, Michalczyk Ł (2017) An integrative description of *Ramazzottius subanomalus* (Biserov, 1985) (Tardigrada) from Poland. Zootaxa 4300: 403–420. https://doi.org/10.11646/zootaxa.4300.3.4

Thulin G (1928) Über die Phylogenie und das System der Tardigraden. Hereditas 11: 207–266. https://doi.org/10.1111/j.1601-5223.1928.tb02488.x

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: 232–235. https://doi.org/10.1093/nar/gkw256

Tumanov DV (2020) Analysis of non-morphometric morphological characters used in the taxonomy of the genus *Pseudechiniscus* (Tardigrada: Echiniscidae). Zoological Journal of the Linnean Society 188: 753–775. https://doi.org/10.1093/zoolinnean/zlz097

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27: 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Vecchi M, Cesari M, Bertolani R, Jönsson KI, Rebecchi L, Guidetti R (2016) Integrative systematic studies on tardigrades from Antarctica identify new genera and new species within Macrobiotoidea and Echiniscoidea. Invertebrate Systematics 30: 303–322. https://doi.org/10.1071/IS15033

Wełnicz W, Grohme MA, Kaczmarek Ł, Schill RO, Frohme M (2011) ITS-2 and 18S rRNA data from *Macrobiotus polonicus* and *Milnesium tardigradum* (Eutardigrada, Tardigrada). Journal of Zoological Systematics and Evolutionary Research 49: 34–39. https://doi.org/10.1111/j.1439-0469.2010.00595.x

White TJ, Bruns T, Lee S, Taylor J (1990) PCR protocols: a guide to methods and application. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zeller C (2010) Untersuchung der Phylogenie von Tardigraden anhand der Genabschnitte 18S rDNA und Cytochrom c Oxidase Untereinheit 1 (COX I). MSc Thesis. Technische Hochschule Wildau, Germany, 105 pp.

Supplementary material 1

***Echiniscus insularis*, MU.001+MU.002**

Authors: Piotr Gąsiorek

Data type: Raw morphometric data

Link: https://doi.org/10.3897/evolsyst.5.59997.suppl1

Supplementary material 2

***Echiniscus tropicalis*, ID.032**

Authors: Piotr Gąsiorek

Data type: Raw morphometric data

Link: https://doi.org/10.3897/evolsyst.5.59997.suppl2

Supplementary material 3

***Echiniscus tropicalis*, ID.939**

Authors: Piotr Gąsiorek

Data type: Raw morphometric data

Link: https://doi.org/10.3897/evolsyst.5.59997.suppl3

Supplementary material 4

***Echiniscus tropicalis*, SG.001**

Authors: Piotr Gąsiorek

Data type: Raw morphometric data

Link: https://doi.org/10.3897/evolsyst.5.59997.suppl4

Supplementary material 5

***Pseudechiniscus mascarenensis*, MU.001**

Authors: Piotr Gąsiorek

Data type: Raw morphometric data

Link: https://doi.org/10.3897/evolsyst.5.59997.suppl5

Supplementary material 6

GenBank accession numbers

Authors: Yevgen Kiosya, Katarzyna Vončina, Piotr Gąsiorek

Data type: GenBank accession numbers

Explanation note: GenBank accession numbers for the sequences used in the present study.

Link: https://doi.org/10.3897/evolsyst.5.59997.suppl6